MANUAL
FOR THE
PHYSIOLOGICAL LABORATORY.

HARRIS & POWER.

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MANUAL FOR THE PHYSIOLOGICAL LABORATORY.

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PREFACE.

It has been customary for several years to issue to the class of Practical Physiology in St. Bartholomew's Hospital papers containing a short account of the histology and chemistry of the various organs, together with the methods employed in the preparation of the tissues for the microscope. The gradual increase in the number of these papers has induced the authors to collect and publish them, as they have found that in a large class it is almost impossible to to teach practical histology without some definite text-book as a basis.

The excellent manuals of Foster and Langley, Schäfer, and Rutherford, possess the objection that they are somewhat too elaborate for the ordinary student, whilst they do not contain any epitome of histology—an omission which in the opinion of the authors greatly detracts from their utility.

This work may therefore prove in some respects more useful to the classes of practical physiology now established in the various medical schools, than those more complete ones which have just been mentioned.

Much of the histology has been taken from the works of Klein, Frey, Ranvier, Stricker, and Schäfer.

At some future time the Authors hope to publish an account of the chief physiological instruments based on the same plan, with the methods for employing them.

The Authors beg to return thanks to Mr. Morrant Baker for many valuable suggestions; to Dr. Klein, F.R.S., for revising part of the histology; to Dr. Russell, F.R.S., for permission to publish the chemical memoranda, compiled under his direction; and to Mr. J. W. Groves, of King's College, for allowing free use to be made of his eminently practical paper on "Hardening and Staining, etc.," recently read before the Quekett Microscopical Society.

VINCENT HARRIS.
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UPPER BERKELEY STREET, W., *March*, 1880.

INTRODUCTION.

It is seldom possible to obtain good results if tissues be examined under the microscope without previous preparation. It has been found necessary, therefore, to use various methods which subserve the purposes of—

(1) *Hardening or Softening,*
(2) *Cutting and Teazing,*
(3) *Staining,*
(4) *Mounting,*

before submitting the structure to examination.

We propose devoting sections to each of these processes before treating of the tissues individually.

Examination of fresh tissues, when necessary, may be done in—

Normal saline solution, which is a ·75% solution of chloride of sodium.

Blood serum.

Aqueous humour of the eye.

Iodised serum, which consists of serum to which a little iodine has been added to preserve it.

1. HARDENING OF TISSUES

is accomplished either by

(1) *Various hardening fluids.*

(2) *Freezing,* a method which is applicable to microtomes only, and of which we shall treat at page 9.

The hardening fluids contain as a rule some of the preparations of chromium, of which the most usual are chromic acid, bichromate of potassium, and bichromate of ammonium.

(1) The solution which is of most general use is a mixture of *chromic acid and spirit;* it is thus prepared:—

℞ Chromic acid $\frac{1}{6}\%$ solution (*i.e.*, containing 1 grm. in 600 cc.), 2 parts.
Methylated spirit, 1 part.

It may be modified by taking equal parts of 0·5% solution of chromic acid, and of methylated spirit. This fluid produces its effects in a very short time—*i.e.*, about seven to ten days.

(2) *Bichromate of potash* is very frequently used in solutions the strengths of which vary from 1% to 5%: the best is the 2% solution, and this, if changed every four days, hardens tissues in a fortnight.

(3) *Bichromate of ammonia* is specially used for hardening the brain and spinal cord, in solutions of 5% strength.

(4) *Müller's fluid* is made thus:—

Potass. bichromat., 2 grms.
Sodæ sulphat., 1 grm.
Aq. destillat., 100 cc.

(5) *Methylated spirit* alone is frequently employed.

(6) *Absolute alcohol* of specific gravity 0·795 is the most rapid hardening fluid. It is not often used, on account of its expense, and because it has a tendency to produce shrinking of the tissue hardened.

Osmic acid, 1%, *gold chloride* 0·5%, *and picric acid* (saturated solution), are sometimes used as hardening re-agents, when the tissue is required to be hardened and stained simultaneously.

Directions for hardening.—Never use anything but fresh tissue. Cut the tissue into pieces, about the size of a hazelnut, with a *sharp* razor. Do not wash with *water*, but if it be necessary to get rid of any foreign body, allow a small stream of a weak solution (·25%) of potass. bichromate to run upon it from a wash-bottle.

Place the cut pieces into a large excess of the hardening re-agent in a stoppered bottle, which should be kept in a cool place.

Change the hardening re-agent frequently—*e.g.*, the chromic acid and spirit solution on the second, fourth, and seventh days.

In all cases, in a week to ten days remove the specimens to spirit to complete the hardening.

Softening fluids.—Certain tissues, especially bone, have to be softened before they can be conveniently cut into sections for the microscope.

The usual fluid is a solution of hydrochloric acid, from 2% to 3%, or a mixture of nitric and hydrochloric acids.

Tendons and other such tissues have to be placed in a solution of potass. bichromat., 2%, in order that the cementing material may be sufficiently softened to allow of teazing with needles.

A saturated solution of picric acid is recommended for softening, and at the same time for preparing teeth.

2. CUTTING.

The most usual way of obtaining thin sections of hardened tissues is by (1) hand-cutting with a sharp razor; but sections may also be cut (2) with machines called microtomes, or (3) with a Valentin's knife.

(1) *Cutting sections by hand.*—In cutting a small piece of tissue it is customary to embed it in a wax mass of some kind. For this purpose small paper boats may easily be formed (*see* Appendix), or a little square tin box with a removable bottom may be used to hold the melted material.

The tissue is held upon a needle mounted in a handle, and is covered over with a layer or two of the melted wax, which is allowed to harden upon it. When the wax mass is cooling, the piece of wax-enveloped tissue is placed in one end of the boat, and is then covered over with more melted wax, and the whole allowed to harden. When cool, the wax is removed from the paper or tin boat, and the tissue is ready for cutting.

Embedding materials :—

(i) Usual form. *White wax and olive-oil*, equal parts.

(ii) *Paraffin-wax and lard*, of each three parts, paraffin oil one part.

(iii) *Cacao butter.*

Various modifications of these may be made: a hard mass must be used in summer, and a softer one in winter.

Razors for hand-cutting must be broad-bladed and hollow-ground: the original "army razor" is the best. They must always be stropped in one direction.

There is no advantage in special section cutters of various forms over ordinary razors.

Directions for cutting by hand :—

Be sure the razor is very sharp.

Hold the razor firmly in the right hand with the fingers closed above the handle, take the wax mass between the index finger and thumb of the left hand, support the back of the razor on the former, and cut from left to right and from heel to tip of razor.

Let the handle be kept in a line with the blade.

Keep the blade well wetted with spirit, into which also the cut specimens must be floated off with a camel's hair brush after each sweep of the razor.

(2) *Cutting sections with microtomes* is of most use when a large number of sections of the same tissue is required.

The chief microtomes are:—

(*a*) *Ranvier's*, which consists of three hollow brass cylinders fitting one inside the other; to the most external a flat circular brass plate is fixed at one end, and to the other a cap which can be screwed on, carrying through its centre a fine screw. The tissue having been embedded in paraffin-wax in one of the cylinders, the cap is adjusted, the microtome held in the hand and the screw turned until the wax is carried up sufficiently high for the embedded specimen to be cut; the flat plate guides the razor. After each section is cut the screw is turned slightly, thereby presenting sufficient of the specimen for another section.

(*b*) *Stirling's* is on the same principle, but is larger, and is fixed to a table by means of a screw. Only one cylinder, however, is fitted to the instrument.

(*c*) *Rutherford's* provides a trough which may be used to contain a freezing mixture of ice and salt. When this is used the tissue is embedded in thick gum, which, on freezing, becomes quite solid, and may be readily cut.

Both (*b*) and (*c*) are generally provided with glass plates instead of brass, to guide the razor.

(*d*) *Williams'* is by far the best microtome for general use. It consists of a circular wooden box about eight or nine inches in diameter. Into the centre is fixed a circular solid brass cylinder, with a brass plate at the top. This box contains a freezing mixture, and is provided with a waste tube to get rid of the water as the ice melts. The cover of the box is wooden, but has a plate of glass fixed upon its upper surface; it also has a central aperture to admit the top of the brass cylinder. This cover is fixed down after the freezing mixture has been placed in the box, and fastened with a lateral screw. The specimen, which should first be left for a couple of hours in water (to get rid of the spirit), is placed on the cylinder plate in a little gum. The gum is soon frozen, and the specimen is fixed by this means. The cutting is done by means of a razor, which is fixed in a movable triangular brass frame, supported on three screws. By the adjustment of these screws the thinness of the section is regulated. The frame works smoothly over the glass table. The upper surface of the razor should be slightly moistened with gum. As the sections are cut they should be swept off the razor with a camel's hair brush, and should be washed in warm water before staining.

There are many other microtomes.

The ice and salt in the case of freezing microtomes should be very finely pounded.

(3.) *Valentin's knife* is sometimes used to cut sections of fresh tissue. It consists of two blades, which can be separated by a screw. The section is cut between them.

Teazing is done by taking a *small piece* of the tissue—*e.g.*, tendon or heart-muscle—in a drop of the fluid in which it is intended to mount the specimen (generally glycerin or saline solution), on a slide, and tearing it in the direction of its fibres with a couple of strong needles mounted in handles to within one-quarter of an inch of their points. If it be stained, put a piece of white paper behind it; if transparent, use some coloured background.

3. Staining Fluids.

Almost all tissues require to be stained with some colouring material in order that their structure may be adequately demonstrated.

The simplest and best is an *aqueous solution of hæmatoxylin*, which is prepared thus :—

℞	Extract of hæmatoxylin . .	60 grms.
	Alum	180 grms.

Rub thoroughly together in a mortar, and add slowly 300 cc. of distilled water; then filter, and to the filtrate add 20 cc. of absolute alcohol. Preserve in a stoppered bottle.

Alcoholic hæmatoxylin is highly recommended by some authorities. Make saturated solutions of crystallized calcium chloride and of alum in proof spirit; mix the solutions in the proportion of one to eight. To this mixture add a saturated solution of hæmatoxylin in absolute alcohol, drop by drop, until the whole becomes a dark purple. This solution may be used at once, but is greatly improved by keeping.

Carmine was formerly used much more than it is at present; it has the disadvantage of staining specimens a colour trying to the eye, whilst it has not the great selective power of hæmatoxylin. Beale's solution is most frequently employed. It is thus prepared:—Dissolve carmine grm. i. in liq. ammoniæ fort. 3 cc. warm, add aq. destillat. 120 cc., and filter. Then add glycerini 30 cc., and spir. vini rectif. 120 cc., and keep in a well-stoppered bottle.

Golding Bird's carmine is made by thoroughly mixing carmine grms. ij and borax grms. viij in a mortar, dissolving in warm water for twenty-four hours. The supernatant fluid which should be decanted is then ready for use.

The carmine staining must be fixed in the same way as the anilin (*vide infra*).

Picrocarmin, or solution of picrocarminate of ammonia, is now much used, especially for "double staining"—*i.e.*, when it is expedient to stain tissues with two colouring materials, in order to bring out certain special features in their structure. It is prepared by adding a saturated ammoniacal solution of carmine to a saturated solution of picric acid until a precipitate forms, evaporating in a water bath to $\frac{1}{5}$ its bulk—filtering and evaporating the filtrate to dryness: a crystallized mass, easily soluble in water, is obtained, which is picrocarmin. The strength of the solution should be about 1% to 3%. During preparation the ammonia should be kept in excess.

Anilin staining fluids are sometimes used, of which the best are—

Rosein—rose red.
Purpurin.
Anilin black.
Anilin blue.

When used, the solution should be weak, and the colour should be fixed by passing the sections through water acidulated with acetic or hydrochloric acid (about 1%), and then transferring rapidly through the spirit and oil of cloves to the preserving medium.

Eosin, a very pretty red dye, is used especially to stain nervous and vascular tissues: a ·01% solution in water is used. Specimens stained with this re-agent should be passed through acidulated water (as above) in order to fix their colour.

Molybdate of ammonium is recommended as producing a cool blue-grey general stain, which does not interfere with the after use of carmine. A 5% solution in water should be used. The specimens are stained under the action of light in twenty-four hours.

Nitrate of silver is used in all cases when it is required to bring out the endothelial cells of serous membranes, as it is taken up by the intercellular substance and reduced as the black oxide under the action of the light, and so maps out the cells in dark lines. The fresh tissue should be plunged into a ·5 to ·25% solution for about fifteen to twenty minutes. After the excess of the salt has been washed away in distilled water, the tissue should be exposed in weak glycerin to the action of sunlight until sufficiently dark.

Chloride of gold is used to stain certain tissues, principally to show the nerves ; it is also used for cartilage—a ·5 to ·25% should be employed.

For a classified list of staining fluids see Appendix.

Directions for Staining.

Use a weak solution of the dye, and allow the sections to remain in it for a long time.

Examine the sections from time to time by transferring to a watch-glass containing spirit, in order to regulate the staining.

Specimens which have been hardened in any preparation of chromium must be passed through a 1% solution of sodæ bicarb. before staining with hæmatoxylin, in order to neutralise the effect of the chromium, after which the sections must be put into two solutions of hæmatoxylin, the first weak, the second stronger.

Preparing for mounting.—Specimens may be mounted at once in glycerin after cutting, staining, and washing in distilled water ; but if it be desired to preserve in Canada balsam or Dammar the sections must pass, after staining, through—

(*a*) Methylated spirit, } to remove the water.
(*b*) Absolute alcohol, }
(*c*) Clove oil, oil of turpentine, or benzol, } to render them transparent.

They must remain in alcohol for five to ten minutes, and in clove oil for the same time.

4. Mounting.

For this process the operator must have—

(1) *Glass slides*, which are slips of glass three inches long and one inch broad, about the thickness of ordinary window glass, with or without ground edges.

(2) *Cover glasses*, which are made of extremely thin glass, circular or square, $\frac{1}{2}$ to $\frac{5}{8}$ inch in diameter.

(3) *Section lifter*, which is easily made by beating out flat one end of a thick copper wire, four or five inches in length. The flattened portion should afterwards be filed at the edges and rubbed smooth with sand-paper.

(4) *Mounting solutions.*—These are: Glycerin, Canada balsam, and Dammar varnish.

Preparation of Canada balsam.—Mix equal parts of Canada balsam and choroform, and warm. The balsam is entirely dissolved. Filter.

Preparation of Dammar varnish.—Dissolve gum dammar in powder 50 grms. in 150 cc. turpentine, and filter; gum mastic 50 grms. in 200 cc. chloroform, and filter. Mix the solutions, and again filter.

Directions for Mounting.

In glycerin.—Sections may be mounted in glycerin direct from water, the cover glass being afterwards painted round with Canada balsam or dammar solution.

In Canada balsam.—Having cut and stained the sections, remove them by means of a needle, and place them one by one in a watch-glass or small dish of absolute alcohol. Leave them in this medium for ten minutes or more, and then pass them into clove oil in another watch-glass. Leave them until perfectly transparent—*i.e.*, for about five or ten minutes. Now take a clean glass slide, and on the centre place a small drop of Canada balsam mixture. Remove the sections from the clove oil by means of the section lifter, and with a needle bring them down into the balsam. Finally, put another drop of balsam on the under surface of a cover glass, and allow it to fall gently upon the slide. *The cover glass must not be pressed down.*

After mounting, label the slides and set them flat in a tray box, made to hold a variable number of specimens from twelve to sixty.

Microscopes.

We append a list of some of the principal *English makers*, from any of whom a useful instrument may be obtained.

Arnold, West Smithfield, E.C.
Baker, Holborn, W.C.
Beck, Cornhill, E.C.
Crouch, Barbican, E.C.
Collins, Gt. Portland Street, W.
Parkes, St. Mary's Row, Birmingham.
Pillischer, New Bond Street, W.
Powell and Leland, Euston Road, N.W.
Ross, New Bond Street, W.
Swift, University Street, W.C.

Foreign Makers:—

Hartnack's (1, Rue Bonaparte, Paris) microscopes are excellent, and the stand of No. VIII. in his catalogue, with oculars Nos. 2 and 3, and objectives (lenses) 4, 7, and 8, is recommended (cost about 250 frs.).

The lenses of Carl Zeiss (Jena) are especially good; A, D, and E are those in ordinary use (cost £1 7s., £2 6s., and £3 11s. respectively).

Verick's microscopes (Paris, 2, Rue de la Parcheminerie, 2) are highly recommended.

ERRATA.

Page 54, lines 8, 11 and 20, *for* '*intra*-lobular' *read* '*inter*-lobular.'

Page 81, line 1, *for* 'reparation' *read* 'preparation.'

Pages 97 and 103, *for* 'quiaicum' *read* 'guiaicum.'

Page 110, line 8, *for* 'The fœtus of' *read* 'The fœtal or.'

Page 120. *Insert* 'interlobular veins,' 54.

Page 121. *Insert* 'Myosin,' 95.

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PART I.

PRACTICAL HISTOLOGY.

Epitome of Apparatus necessary for this work.

Microscope.
A pair of fine scissors.
A pair of fine forceps.
Two scalpels.
Needles mounted in handles.
Razor.
Glass slides and cover glasses.
Watch glasses (6).
Section lifters (2).
Labels.
A box or cabinet for mounted specimens.

Reagents—(the most used).

Bichromate of potassium 1°/o & 2°/o solution.
Bichromate of ammonium 5°/o ,,
Müller's fluid
Chromic acid $\frac{1}{6}$°/o ,,
Osmic acid, Auric chloride, and Silver nitrate.
Methylated spirit.
Absolute alcohol.
Clove oil.
Hæmatoxylin solution.
Picrocarmin ,,
Carmine.
Anilin colours.
Eosin.
Sodæ bicarb., 1°/o solution.
Acetic acid, ,,
Saline solution.
Tannic and Boracic acids.
Glacial acetic acid.
Chloride of sodium.
Glycerin.
Dammar varnish.
Canada balsam.
Distilled water.

THE BLOOD.

HUMAN BLOOD.

Take a small drop of blood from the finger; place it on a perfectly clean glass slide, and put over it a glass cover. Examine with magnifying power of 200 or 300. (Hartnack., Oc. 3, Obj. vii.)

Coloured Corpuscles :

Circular discs $\frac{1}{3000}$ — $\frac{1}{3500}$ inch in diameter; and $\frac{1}{10000}$ inch in thickness, depressed a little on each side. When seen sideways, biconcave or dumb-bell shaped. Of a pale buff colour, but when aggregated of a reddish tint. They have a tendency to run together, collecting in rolls or rouleaux. Notice a corpuscle as it rolls over, and observe the change in its form, being alternately circular and biconcave.

The corpuscle has no nucleus; the false appearance of a nucleus is occasioned by the refraction of light in passing through a biconcave disc. Prove this by slowly altering the focus. The corpuscles consist of two parts : a stroma, which is colourless, and the coloured part, a red crystallizable substance, *hæmoglobin.*

Colourless Corpuscles :

Their proportion to the coloured varies from 2 to 10 in 1,000, the number being greater after taking food.

When perfectly fresh, they are spherical and faintly granular ; they quickly alter and become markedly granular.

About $\frac{1}{2500}$ inch in diameter; nucleated, the nucleus not often apparent without the addition of weak acetic acid. They are nearly always isolated, and do not collect together or mix with the coloured discs. They are endowed with the power of spontaneous motion (amœboid movement). This is best seen with a "warm stage."* Observe and draw the changes in form of a colourless corpuscle at intervals of five minutes.

Action of Reagents on the Blood :

Take a small drop of blood on a slide and add to it a drop of saline solution. Cover it with a glass cover ; any reagent may now be made to act upon the blood by placing a drop of it on one side of the slip and applying a piece of filter paper to the opposite side. A stream of the fluid passes under the cover glass. This is called *Irrigation.*

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* See Appendix.

Irrigate specimens of blood with (1) *Water:* the red corpuscles become smooth and pale, and disappear.

(2) *Acetic acid:* the same changes take place, the colourless corpuscles become more distinct and their nuclei more apparent.

(3) *Tannic acid:* the hæmoglobin collects in small lumps at the sides of the red corpuscles. (The action of carbonic acid gas, chloroform vapour, and certain other reagents, will be found in the Appendix.)

HÆMIN CRYSTALS:

A drop of blood is dried on a glass slide; two or three granules of common salt are added. With a capillary pipette add a drop of glacial acetic acid and then cover. Raise the temperature gradually to the boiling point over a spirit lamp until the greater part of the acid has evaporated. A number of small reddish-brown rhomboidal crystalline plates are seen.

HÆMOGLOBIN CRYSTALS:

Take a drop of blood from a guineapig and let it coagulate on a slide; add a little water and take up the clot with the forceps, and let several small drops fall upon another slide. As these evaporate, hæmoglobin crystals of various sizes shoot out from the edges, separately and in bundles.

BLOOD-CORPUSCLES OF OTHER VERTEBRATE ANIMALS.

COLOURED CORPUSCLES:

In nearly all Mammalia the coloured corpuscles are round disc-like non-nucleated bodies, similar to those of man, but differing in size. In this respect they vary considerably.

In Birds, Reptiles, Amphibia, and Fishes, the corpuscles are oval and nucleated, the nucleus presenting a central elevation on each surface. These corpuscles are larger in birds than in mammalia; still larger in fishes; and of a yet greater size in amphibia.

COLOURLESS CORPUSCLES:

The general characters of these corpuscles are similar in all animals, but they are found in much larger proportion in the blood of fishes and amphibia than in that of mammalia and birds.

Irrigate a specimen of newt's blood with boracic acid 1°/₀. The hæmoglobin collects around the nuclei of the red corpuscles.

EPITHELIUM.

SQUAMOUS EPITHELIUM.

(*a*) From the Mouth:

With a blunt knife, or with the finger nail, scrape off a thin shred from the mucous membrane of the cheek; mix with a drop of normal saline solution on a slide; place on it a cover glass, and examine with a power of about 200 diameters.

The cells consist of large, flat, roundish, or irregularly polyhedral bodies, of various sizes. The substance is more or less transparent, containing granular matter. The nuclei small, oval, frequently granular, and sometimes missing.

(*b*) From the Œsophagus:

Tease a scraping from the œsophagus of a cat or dog in a small drop of glycerin. The œsophagus should be placed in potass. bichrom. 2°/₀ for twenty-four hours, and then, after washing, should be left in hæmatoxylin solution for several hours. The nuclei of the cells are thus stained.

GLANDULAR EPITHELIUM.

From the Kidney or Liver:

Take a scraping from the freshly cut surface of a kidney or liver; prepare in salt solution.

The cells vary in shape and size, are pale, and fairly well defined.

COLUMNAR EPITHELIUM.

From the Intestine:

Take a small portion of the intestine of some animal (cat, rabbit, or dog), place it in a 2°/₀ solution of bichromate of potash for twenty-four hours or more; detach a fragment of the mucous surface by scraping lightly with a knife, and tease up with needles in a drop of saline solution or glycerin on a glass slide.

The cells are cylindrical or conical in form, with fairly well-defined outline; protoplasm finely granular; nucleus clear, oval, well-defined.

When an aggregation of cells is seen from above, as on the surface of a villus, it has the appearance of a regular mosaic.

TRANSITIONAL :

Best seen in the bladder, which may be prepared in the same way as the œsophagus. The shape of the cells should be noted: some are tailed, others concave on one side, spindle-shaped, or caudate. The nuclei are very large.

CILIATED EPITHELIUM.

Scrape lightly the mucous surface of a prepared trachea, and tease out in glycerin, and examine in a similar manner.

The cells have cilia at one end and a tail at the other, with a large nucleus near the tailed end.

Study of Ciliary Motion.

With a sharp pair of scissors cut off a small fragment of one of the branchiæ of a living oyster or mussel. Put it upon a glass slide in a drop of salt solution, and bring it under the microscope as quickly as possible (Hartnack, Oc. 3, Obj. vii.).

Ciliary movement at first very rapid ; soon becomes slower, and finally ceases.*

PIGMENT :

Can be studied in scrapings from the choroid, iris, etc. They are either branching dark irregular cells with clear nuclei, or flattened polygonal cells.

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* See Appendix.

ENDOTHELIUM.

ENDOTHELIUM OF SEROUS MEMBRANES.

Preparation—The Silver Method.

From an animal which has been recently killed by bleeding, take a portion of the omentum, pericardium, mesentery, or other serous membrane. Immerse it at once in a $\frac{1}{4}$°/o solution of nitrate of silver. Leave it for about ten minutes. Then wash thoroughly in water, and expose to the light for about a quarter to half an hour: that is, until it has assumed a brownish colour. Cut the membrane in small pieces with scissors, and mount in glycerin. *(Vide infra.)*

The endothelial linings of arteries and veins may be demonstrated in a similar manner. To show the endothelium of the lymphatic vessels, kill a rabbit rapidly by bleeding, open the thorax, lift up the lungs and heart, rub the tendon of the diaphragm briskly with a camel's-hair brush wetted in saline solution. Pour upon the tendon the solution of nitrate of silver, and allow it to remain for ten minutes, then remove the tendon carefully, wash in distilled water, cut in pieces, expose to the light, and mount in glycerin with the brown surface uppermost.

The Glycerin Process.

The membrane is to be placed in a small glass dish partly filled with distilled water. Take a clean glass slide, and immerse it in the water. Then float the specimen on to the centre of the slide, taking care to prevent any folding of the tissue. Remove the slide from the water, and arrange the specimen with needles. Take away the superfluous water with strips of filtering paper. Put a drop of strong glycerin on the under surface of a cover glass, and allow it to fall gently on the slide so as to form a thin layer between the glasses. Finally paint round the edge a layer of Dammar varnish.

General Characters of Cells.

On examination, the surface of the membrane is found to consist of a single layer of flattened polyhedral cells, variously modified, and forming a mosaic. Nuclei generally single, and only to be detected in deeply stained specimens, appear as bright and almost colourless oval bodies within the cells. When seen in profile, the nuclei occasion a projection from the surface.

THE CONNECTIVE TISSUES.

VARIETIES.

1. White fibrous tissue.
2. Elastic tissue.
3. Areolar tissue.
4. Gelatinous or embryonal tissue.
5. Adipose tissue.
6. Cartilage.
7. Bone.

I. WHITE FIBROUS TISSUE.

PREPARATION.

From a newly killed young rat or mouse the tail is cut off close to the base. The skin is removed, and a small piece of the extremity is pinched off between the nails, and is drawn away from the rest of the tail. In separating this piece a number of fine threads, the tendons, will be noticed. One of these of moderate size is selected and teased out in glycerin.

STRUCTURE.

The tissue is then seen to consist of parallel *bundles of fibres*, which vary in thickness, and are held together by an homogeneous and albuminous cement substance. The individual fibres forming the bundles are straight or wavy, and are extremely delicate. Acetic acid added to tendons causes *the fibres to swell up* and to disappear, owing to the presence in the tissue of a substance which is readily convertible into glutin or gelatin. The bundles of fibrils are surrounded by a more or less complete sheath of elastic tissue, which is not acted upon by dilute acids; hence the constricted appearance seen in tendons to which acetic acid has been added.

To demonstrate the presence of THE TENDON CORPUSCLES.

PREPARATION.

The most delicate of the tendons obtained from the tail of a rat is stretched, whilst it is still perfectly fresh, upon a glass slide. The extremities of the tendon are allowed to dry, and by this means it is maintained in an extended condition. A few drops of picrocarmin are placed upon the centre of the tendon, and are washed away with distilled water after the expiration of half an hour. A drop of glycerin acidified with acetic or formic acid is then added, a hair is placed by the side of the tendon to obviate pressure, and a cover glass is put on, the preparation being sealed up in the usual way. Good results can also be obtained by mounting the isolated tendons in a 1°/₀ solution of acetic acid to which $\frac{1}{3}$ its volume of logwood alum solution has been added. The preparation must be examined as soon as possible (Schäfer). Also by treatment with a 0·1°/₀ solution of osmic acid for an hour, washing in distilled water for three hours, and subsequent staining with picrocarmin.

STRUCTURE.

On examination the tendons thus treated are found to consist of parallel bundles of fibres, arranged in groups whose substance is almost colourless. Between each two groups is a lymph channel, in which lie nearly parallel layers of delicate stained cells—*the connective tissue* or *tendon cells*—forming for each channel a single continuous row of rectangular plates. Each plate is provided with a more deeply staining nucleus. The cells are separated from each other by a cementing substance, and they possess fine processes. Each cell presents a straight ridge—*the elastic stripe.* This ridge is formed by the union of two or three concave portions of which the cell is composed, to enable it to adapt itself to the curved surfaces of the tendon bundles. The lymphatics may be demonstrated by staining the tail of a very young rat in chloride of gold, and then making fine transverse sections; dark masses will then be seen in the tendon corresponding to the *lymphatic channels* filled with an albuminous fluid plasma. Radiating from these masses are fine septa—*the cement substance*—binding together the contiguous bundles.

II. ELASTIC TISSUE.

PREPARATION.

Tease out a small piece of the ligamentum nuchæ of an ox in glycerin and examine.

STRUCTURE.

Elastic fibres are *thick* and well defined; they do not form bundles; they *branch* dichotomously, and *anastomose* with each other to form a real network; when torn they *curl up* at the ends. They do not swell up when treated with acids, and they yield elastin.

III. AREOLAR TISSUE.

PREPARATION.

This form of tissue is best seen in specimens of intestine and skin. A small artificial bulla is formed in a rat or rabbit which is still warm by the injection into the subcutaneous tissue of a 0·2% solution of nitrate of silver or osmic acid, which is allowed to remain for ten to thirty minutes. The bulla is then opened with a pair of fine curved scissors, and the delicate subcutaneous tissue is rapidly removed and spread out on a glass slide. It is immediately covered with a thin glass, and the preparation is stained for twenty-four hours with picrocarmin. Glycerin is passed through until all the superfluous staining material is removed, after which the preparation is sealed up.

STRUCTURE.

The tissue is composed of delicate bundles of *ordinary white fibrous tissue*, some of the fibres are fibrillated, and all interlace with each other ; the meshwork thus formed contains a few very fine fibrils of *elastic tissue*. The interspaces are filled with lymph-containing *lymph corpuscles*. Large *plate-like cells* which appear to lie upon the surface of the bundles of fibres are also seen. When viewed sideways these cells have a branched appearance, and form the *plasmatic cells*. *Fat cells* are also present.

IV. GELATINOUS, EMBRYONAL, MUCOUS, OR WHARTON'S TISSUE.

PREPARATION.

Present in the fœtal umbilical cord, and in the fœtal skin. The connective tissue is obtained from a bulla formed by the injection of a dilute solution of gold chloride, in a stronger solution of which it is subsequently stained.

STRUCTURE.

A transparent jelly-like substance in the youngest condition, containing a hyaline mucous substance within a reticular framework. At a later period *bundles of fibrous connective tissue* are apparent, as well as *branched cells*, *blood-vessels*, and *fat cells* in an early stage of development. The tissue yields mucin on boiling. *The vitreous humour* appears to be a variety of this tissue in which the branched cells have lost their processes.

V. ADIPOSE TISSUE.

PREPARATION.

Tease out a small piece of fat in glycerin. Leave a small piece of fat, which has been partially teased, in ether for twenty-four hours, and the fatty portion will be dissolved out. Examine the preparation of areolar tissue formed by the injection of nitrate of silver.

STRUCTURE.

Adipose tissue consists of a matrix or network of areolar tissue containing fat cells. *Fat cells* are clear, well defined, rounded vesicles of varying size, filled with an oily fluid, which often gives rise after death to *crystalline needles*, probably of margarin, radiating from the centre of the cell. In successful preparations a fine zone of *protoplasm*, with a *nucleus* at one pole, can be seen surrounding the cell more or less completely. The fat cells may either form compact masses, with only a small amount of connective tissue, or they may be more or less isolated. The tissue possesses a capillary network of *blood-vessels.* Between the fat cells flattened nucleated *connective-tissue cells* may be demonstrated.

CARTILAGE.

CARTILAGE consists of two parts:

1. Cells.
2. Matrix or Inter-cellular material.

According to the nature of the matrix, cartilage is classed as—

1. Hyaline cartilage.
2. Fibro-cartilage.
3. Elastic cartilage.

I. HYALINE CARTILAGE.

PREPARATION.

Hyaline cartilage consists of several kinds: costal, tracheal, articular (from the articular surface of bone), ossifying or intermediary, and embryonal. Portions of each of these cartilages should therefore be examined. The cartilages may be hardened in a solution of chromic acid 1 in 600, in a saturated solution of picric acid, or by the gold method. In every case the sections, which must be very thin, should be stained with carmine or hæmatoxylin.

STRUCTURE.

All cartilage, with the exception of the free extremity of articular cartilage, possesses a delicate vascular connective-tissue sheath—*the perichondrium. The cartilage cells* are spherical or oval protoplasmic bodies, generally containing a single nucleus. The cell protoplasm forms a fibrillar meshwork which is contracted in embryonal and articular cartilages. Each cell is placed in a lacuna, enclosed by a firm, structureless but transparent *matrix*, yielding chondrin. In growing cartilage a special layer—*the limiting membrane*—can be distinguished between the lacuna and the ground substance. In some cases a single lacuna may contain more than one cell due to reproduction of the cartilage cell by fission: and the various stages in the division of cells may often be well seen. Near the perichondrium the cartilage cells become flattened and smaller; near the articular surface they are branched: in ossifying cartilage they are arranged in parallel rows. The matrix possesses an anastomosing system of lacunæ and canals in connection with the *lymphatic* system.

II. FIBRO-CARTILAGE.

PREPARATION. As for hyaline cartilage.

White fibro-cartilage occurs in the intervertebral substance and in sesamoid bones.

STRUCTURE.

It consists of groups of slightly flattened elastic *cells*, each with a round nucleus, and enclosed in a distinct capsule. The matrix is composed of bundles of fibrous tissue, which sometimes form lamellæ with occasionally a concentric arrangement.

III. ELASTIC CARTILAGE.

PREPARATION : As for hyaline cartilage.

This form of cartilage occurs in the lobe of the ear, in the epiglottis, in the cornicula laryngis, in the cartilages of Wrisberg and Santorini, and in the Eustachian tube.

STRUCTURE.

Elastic cartilage in the adult is hyaline cartilage permeated by elastic fibrils. *The fibrils* are arranged so as to form the trabeculæ of a reticular framework; they branch and anastomose very frequently. The meshes contain fusiform groups of large nucleated *cells*, surrounded by a larger or smaller amount of hyaline cartilage substance.

BONE.

PREPARATION.

The fresh bones of any small animal, well cleared of the surrounding tissues, are to be placed for two or three weeks in a large quantity of $\frac{1}{2}{}^{\circ}/_{\circ}$ solution of chromic acid, containing five drops of hydrochloric or nitric acid to each ounce of the solution. When the whole of the earthy matter is dissolved out, sections are to be cut with a razor in various directions and examined in glycerin.

GENERAL CHARACTERS.

In transverse sections of the compact tissue of long bones are seen Haversian systems, more or less perfect, and Haversian interspaces. Each system consists of the central *Haversian canal*, which is generally round or oval, with an average diameter of $\frac{1}{500}$ in., and is lined with a delicate membrane continuous with the periosteum, surrounded by concentric lamellæ of bone, in which are the lacunæ and canaliculi. *Lacunæ* $\frac{1}{2500}$ in. in length, generally well marked, contain shrunken bone corpuscles. *Canaliculi* usually indistinct, but when seen plainly forming a complete system of communication between the lacunæ of the same and neighbouring Haversian systems and interspaces. They contain in the fresh condition prolongations from the bone corpuscles. Each Haversian system is more or less isolated from its neighbour by a layer of bone which contains but few canaliculi. The *Haversian interspaces* are the portions of bone filling up the interval between one or more of the circular Haversian systems. They do not contain any central canal ; their general characters are otherwise similar to the systems.

In longitudinal sections the Haversian canals which run longitudinally are seen to anastomose freely by transverse or oblique channels. The lacunæ and canaliculi present much the same characters as in transverse sections. The Haversian canals which run near the circumference of the bone may open on the outer surface so as to admit blood-vessels from the periosteum, whilst those opening into the medullary canal receive blood-vessels, and in the case of the larger ones medulla from the interior of the bone.

In preparations of calcified bone it may be seen that the lamellæ are bolted together by *the perforating fibres of Sharpey*.

Bone situated immediately beneath an articular cartilage differs in not possessing Haversian canals, in the lacunæ being three or four times larger than in ordinary bone, and in being destitute of canaliculi. *The periosteum* covering the free surface of bones consists of an external layer of dense fibrous tissue supplied by capillary blood-vessels, and an internal osteo-genetic layer containing a plexus of delicate connective-tissue fibrils; in the meshwork formed by these fibrils are capillary blood-vessels and a number of nucleated cells. *The medulla* is of the yellow kind, and is chiefly composed of fat cells, with intervening membranes of flattened connective-tissue cells; it also contains numerous cells possessing one or two nuclei.

In spongy bones—*e.g.* a vertebral, carpal, or tarsal bone—the tissue consists of bone-trabeculæ, forming a more or less open framework, in which is embedded the medullary substance. Bone-trabeculæ contain lacunæ with bone-corpuscles and ill developed canaliculi. The medullary substance is of the red kind; it is rich in blood-vessels, and in cells having the characters of lymph-corpuscles, fat-cells, &c.

THE DEVELOPMENT OF BONE.

(*a*) In Cartilage.

(*b*) In Membrane.

(*a*) Bone developed in cartilage or endochondral bone. Stage i. *Hyaline cartilage* covered by perichondrium. *The perichondrium* consists of an outer layer of embryonal connective tissue, and an inner osteo-genetic layer containing spherical cells—the future osteoblasts, and blood-vessels. Stage ii. *The inner layer of perichondrium penetrates the cartilage*, forming for itself channels by absorption, and carrying with it blood-vessels and cells. The growth of the perichondrium inwards starts at the centres of ossification. Stage iii. *The primary marrow cavities* are formed by the appearance of lacunæ near the cartilage channels, which then become confluent; whilst the trabeculæ separating neighbouring lacunæ become calcified. The primary marrow filling the marrow cavities is the periosteal ingrowth containing the vessels and cells. Stage iv. The calcified trabeculæ after becoming ensheathed with osseous material, are absorbed. A network of osseous trabeculæ instead of a network of calcified cartilage is thus formed, whilst the whole tissue resembles spongy bone. The surfaces of the osseous trabeculæ are covered with osteoblasts, whilst the cavities separated by the trabeculæ are filled with marrow rich in vessels and cells. Stage v. The endochondral spongy bone is absorbed, from the centre outwards; the large *medullary cavity* is thus formed. *Bone from the periosteum* is also developed round the endochondral bone. *The osteoblasts* multiply and become converted into the osseous matrix and into bone-corpuscles. The meshes of the spongy periosteal bone are *the Haversian spaces;* they contain marrow from which a series of concentric lamellæ are formed. The spaces are thus gradually reduced to *Haversian canals.* Stage vi. All the endochondral bone is absorbed, and the ossified trabeculæ are represented by the interstitial substance separating the concentric Haversian lamellæ.

(*b*) Intramembranous. The membrane corresponds to the future periosteum ; it consists of two parts as above. Stage i. The cells of the osteo-genetic layer—*the osteoblasts*—increase and form the osseous matrix by excreting ossein around them and the bone-corpuscles, thus forming ossified trabeculæ which start from the centres of ossification. Stage ii. Portions of the trabeculæ are absorbed (*osteoporosis*), whilst, as in endochondral bone, concentric lamellæ are formed by the marrow in the Haversian canals.

The formation of intramembranous bone is identical with the formation of periosteal bone. The absorption of osseous substance is in nearly every case associated with the presence of multinucleated giant cells—*the osteoclasts.*

MUSCULAR TISSUE.

VARIETIES.

1. Striated or striped.
2. Plain or unstriped.

PREPARATION.

For the examination of fresh muscle, tear away a small fragment of the tissue with a pair of forceps from a recently-killed cockroach or water-beetle (*Dytiscus marginalis*), and at once tease it up thoroughly in a drop of salt solution or water on a glass slide. Examine as soon as possible.

Muscle is also prepared by taking small pieces of fresh tissue from the voluntary muscles and the heart, as well as from any of the viscera containing unstriped fibres, and soaking them for a few weeks in a $2^{\circ}/_{\circ}$ solution of chromic acid, or in a $\frac{1}{10}{}^{\circ}/_{\circ}$ osmic acid for one hour, then wash in distilled water for two hours. A fragment of the tissue is removed, and, having been well teased with needles, is examined in water, glycerin, or other medium.

Unstriped tissue is prepared by distending a piece of rabbit's intestine with saline solution, leaving it in a $1^{\circ}/_{\circ}$ solution of anilin black for twenty-four hours, and stripping off the outer coat with forceps. Small pieces of this coat are then to be mounted in glycerin.

GENERAL CHARACTERS.

STRIPED MUSCULAR TISSUE.

From Voluntary Muscle.—Consists of long *fibres*, which are cylindrical, but appear in transverse section as rounded polygons. Each fibre is made up of a number of exceedingly fine and delicate filaments, *the fibrillæ*, enclosed within the sarcolemma. The fibres are aggregated into *bundles;* several bundles forming *fasciculi*, and these the anatomical *muscle*. *Perimysium* or fibrous connective tissue surrounds the bundles; from it pass off small processes of connective tissue, with cell plates and plasma cells, between the muscle fibres—*the endomysium.* Each fibre consists of broad dim bands of highly refractive substance representing the contractile portion of the muscle fibre—*the contractile discs*—alternating with narrow bright bands of a less refractive substance—the *interstitial discs.* After hardening, each contractile disc becomes longitudinally striated, the thin oblong rods thus formed being the *sarcous elements* of Bowman. The sarcous elements are not the optical units, since each consists of minute doubly refracting elements—

the disdiaclasts of Brücke. When seen in transverse section the contractile discs appear to be subdivided by clear lines into polygonal areas—*Cohnheim's fields,* each corresponding to one sarcous element prism. The clear lines are due to a transparent interstitial fluid substance pressed out of the sarcous elements when they coagulate. *The sarcolemma* is a transparent structureless elastic sheath of great resistance which surrounds each fibre. From the sarcolemma transverse membranous septa—*the membranes of Krause*—extend inwards across the muscle at regular intervals. By these septa the muscle fibre is divided into equal-sized *muscle compartments*, each containing one contractile disc. The membranes of Krause are so placed that each passes across the middle of an interstitial disc, which is thus divided into two *lateral discs.* A thin transverse median disc—*the disc of Hensen*—is occasionally seen to divide the contractile disc. In some fibres, chiefly those from insects, each lateral disc contains a row of bright granules forming *the granular layer* of Flögel. The fibres contain nuclei, which are roundish, ovoid, or spindle-shaped in different animals. These nuclei are situated close to the sarcolemma, their long axes being parallel to the fibres which contain them. Each nucleus is composed of a uniform network of fibrils, and is embedded in a thin more or less branched film of protoplasm. The nucleus and protoplasm together form the muscle cell or *muscle corpuscle* of Max Schultze.

Muscular Tissue of the Heart.—Fibres very small and finely striated. Striæ generally indistinct, often showing only as coarse granules. There is no sarcolemma. Many of the fibres anastomose and branch.

NON-STRIPED MUSCULAR TISSUE.

Made up of bundles of cells, bound together by an albuminous cementing substance—the *endomysium*—in which lie connective-tissue cells and a few fibres. The *perimysium* continuous with the endomysium is the fibrous connective tissue surrounding and separating the bundles of muscle cells. Fibres fusiform, band-like, or spindle-shaped, containing elongated or staff-shaped nuclei, placed midway in the fibres. Each *muscle cell* consists of a fine sheath, probably elastic ; of a central bundle of fibrils representing the contractile substance ; and of an oblong nucleus, which includes within a membrane a fine network anastomosing at the poles of the nucleus with the contractile fibrils. Ends of fibres usually single, sometimes divided. There is no sarcolemma.

NERVE TISSUE.

VARIETIES.

1. Nerve fibres { Medullated. / Non-medullated.
2. Nerve cells.

PREPARATION.

Cut longitudinal sections of a portion of a sciatic nerve, which has been hardened for about ten days in a 2°/₀ solution of bichromate of potassium, or a mixture of spirit and ¼°/₀ chromic acid in equal parts. Stain these deeply in hæmatoxylin solution, and tease them out, then mount in the ordinary way in Canada balsam. Cut also transverse sections, stain, prepare and mount in similar manner.

GENERAL CHARACTERS.

1. NERVE FIBRES.

(*a*) *Medullated Nerve Fibre.*

FROM SPINAL NERVES.—The nerve trunks are composed of a variable number of bundles (*funiculi*) of nerve fibres which have a special sheath (*perineurium* or *neurilemma*) and are enclosed in a firm fibrous sheath (*epineurium*), which also sends in processes of connective tissue, connecting the bundles together. In the funiculi between the fibres is a delicate supporting tissue (the *endoneurium*).

Each nerve fibre is made up of the following parts :—

(1) *Primitive nerve sheath*, or *nucleated sheath of Schwann.* (2) *Medullary sheath*, or *white substance of Schwann.* (3) *Axis cylinder*, *primitive band*, *axis band*, or *axial fibre.*

Schwann's sheath, which is the external layer of the fibre, appears to be a simple, transparent, colourless, homogeneous structure, with a varied number of oval nuclei attached. It probably consists however of nucleated endothelial cells joined end to end and forming a complete membrane.

The *medullary sheath*, the middle layer, which forms the greater part of the nerve, surrounds the axis cylinder, and has a double contour ; and at regular intervals are constrictions in the medullary sheath (Ranvier's constrictions), caused by the thinning or interruption of the medullary substance.

The *axis cylinder* is situated in the middle, and appears as a faintly marked band, with an exceedingly fine and even outline, and is made up in the prepared specimen of a number of most delicate fibrils (*primitive fibrils*).

From Cerebro-spinal Centre, the Optic and Auditory Nerves.—The general appearances are the same, excepting that there is no sheath of Schwann.

(*b*) *Non-medullated Nerve Fibre.*

From Sympathetic and Olfactory Nerves.—Consist of simple filaments forming an axis cylinder, and surrounded by a nucleated sheath of Schwann.

2. Nerve Cells.

(Are examined with the brain and spinal cord.)

NERVE TISSUE.—PAPER II.

SPINAL CORD.

Preparation.—Small lengths (about $\frac{1}{4}$ to $\frac{1}{2}$ inch) of the spinal cord of the cervical, dorsal, and lumbar regions of a calf, sheep, or pig, should be hardened in $5^{\circ}/_{0}$ solution of bichromate of ammonium for a week, and should then be transferred to spirit —or the usual mixture of chromic acid and spirit may be used. A solution of eosin is recommended by some for staining the sections, but carmine or picrocarmin and hæmatoxylin act equally well. Anilin black $1^{\circ}/_{0}$ solution stains the ganglion cells excellently.

Structure consists of (1) *white*, and (2) *grey matter*, supported by fine connective tissue (*neuroglia*).

(1) *White*, situated externally and forming the greater portion of the cord, is most marked in the dorsal region, and then in the cervical; it is made up of longitudinal fibres from $\frac{1}{1500}$—$\frac{1}{15000}$ inch, which are finer in the posterior and postero-lateral columns than elsewhere, of some transverse fibres in the anterior white commissure, and of a few nerve cells.

(2) *Grey*, forms the interior of the cord, and on transverse section presents two crescentic masses with concavities outwards, joined across the middle by a transverse piece (posterior grey commissure). In the centre is a small canal lined with columnar ciliated epithelium.—The crescents present (*a*) anterior horn (cornu), short and thick, extending towards the attachment of the anterior roots; (*b*) posterior horn, longer and more slender. In the concavity of each crescent the grey matter sends out processes which enclose portions of the white substance.

The grey matter is made up of small, non-medullated fibres which chiefly form a dense network, continuous with the roots of the nerves; part of these fibres are derived from the branches of the nerve-cells, which are embedded in the network. These cells are of two kinds:—(1) *Large, branched, and nucleated*, which are chiefly to be found in the anterior cornua, especially at their upper and outer parts, but also at the inner part of the base (cervix cornu) of the posterior horn, forming the *posterior vesicular column*, which is best marked in the lumbar enlargement of the cord; and, lastly, in the concavity of the crescent is a group of cells, occupying a projection of grey matter there (tractus intermedio-lateralis), which exists chiefly in the dorsal region. (2) Smaller cells scattered throughout the grey matter, but chiefly at the tip (caput cornu) of the posterior cornu, in a finely granular basis, and among the posterior root fibres (*substantia gelatinosa cinerea* of Rolando).

ORIGIN OF THE SPINAL NERVES.

(*a*) *Anterior roots*, pass into the anterior cornua, and are there distributed thus :—(1) Some fibres pass backwards, and form connections with fibres from elsewhere ; (2) Some spread obliquely upwards and downwards ; (3) Some pass externally to the lateral columns ; and (4) Others internally cross to the other side in the anterior white commissure.

(*b*) *Posterior roots*, enter the posterior cornua, either at the tip, through the substantia gelatinosa, or by the inner side. Those which enter at the tip, as a rule, turn upwards or downwards : some reaching the anterior cornua ; and the others, the opposite side, through the posterior grey commissure. Of those which enter by the inner side of the cornua—(1) Some pass at once into the grey matter ; (2) Others through the posterior vesicular columns ; (3) The majority pass up (or down) in the white substance of the posterior columns, and enter the grey matter at various heights ; (4) Not a few are lost in the posterior white columns.

NERVE TISSUE.—Paper III.

Cerebrum.

Preparation.—Place small pieces from different parts of the cerebrum in a $2^{\circ}/_{\circ}$ solution of bichromate of ammonia for two days, after which transfer to weak, and finally to strong spirit. Care must be taken to get vertical sections. Stain in anilin blue-black.

Structure.—The cerebral convolutions are divisible into (*a*) Cortical grey portion, and (*b*) White medullary substance. (*a*) *Cortical grey portion* is composed of—(1) *An external layer* containing a few small cells with fine processes embedded in a considerable quantity of neuroglia. This layer composes about $\frac{1}{10}$ of the whole thickness of the grey substance. (2) *The second layer* of small, densely aggregated, pyramidal cells, provided with branching processes. This layer is of nearly the same extent as the previous one. (3) *The third layer* is of greater width, and is somewhat paler than the first and second layers; it is composed of large and small pyramidal cells, arranged with their apices turned towards the surface of the convolution. The larger cells average $\frac{1}{1800}$ in. across their base. The cells are arranged in groups, and are separated from each other by bundles of radiating nerve fibres, each bundle being about $\frac{1}{1500}$ in. in diameter. The pyramidal cells send downwards three processes, of which the middle one forms an axis cylinder. Both cells and processes are striated longitudinally, and generally contain a little yellowish pigment. (4) *The fourth layer* is somewhat narrower than the preceding; it consists of small, irregularly-placed, granule-like corpuscles, with delicate processes. The cells are less distinctly separated into groups. (5) *The lowest layer* is of considerable width; it contains, in addition to cells resembling those of the fourth layer, fusiform cells arranged vertically at the summit of a gyrus, but parallel to the surface of a sulcus. This layer gradually blends with (*b*) *The white substance,* composed essentially of white nerve fibres, which are smaller than those of the spinal cord, with an average diameter of $\frac{1}{10000}$ in. In the neighbourhood of the cortex, a few non-medullated fibres can be seen. (*c*) *The neuroglia,* formed of a homogeneous matrix, in which lie numerous elastic fibrils, connected into a network. With this network, the branched nucleated cells—of Deiter—are connected.

CEREBELLUM.

Preparation.—As for cerebrum. Stain in anilin blue.

Structure.—Like the brain, it is divisible into (*a*) Cortical grey; and (*b*) Internal white substance. *The Cortex* is divisible into—(1) *The Molecular layer*, the most external, consisting of a nerve network containing small pear-shaped multipolar ganglion cells. The fibres of the network in the more superficial portions are nearly vertical to the surface; they are derived partly from the neuroglia, partly from the processes of the cells of Purkinje. (2) A single layer of large spindle-shaped ganglion cells $\frac{1}{800}$ — $\frac{1}{1000}$ in. in diameter, *Purkinje's cells.* Each cell possesses one branched process which extends into the molecular layer, where it branches dichotomously, some of the finest ramifications looping backwards to terminate in the granular layer, and an unbranched axis cylinder process passing downwards. The cells lie in a pericellular space, and each consists of a minute network of fibrils extending into the branched processes. The nucleus is spherical and oval. (3) *The granular layer*, containing a network of minute fibrils, and dense groups of granule-like corpuscles. These corpuscles average $\frac{1}{4000}$ — $\frac{1}{2500}$ in. in diameter. (*b*) *The medullary centre* or *internal white substance* consists of nerve fibres arranged in parallel or interlacing bundles.

The neuroglia of the white matter contains rows of small cells, each with a spherical nucleus, between bundles of nerve fibres.

The blood-vessels of the grey matter pass from the pia mater in a vertical or oblique direction, and anastomose into a uniform network. The blood-vessels of the white matter form a network with longitudinal meshes. The vessels lie in lymph channels, *the perivascular lymphatics* of His.

BLOOD-VESSELS.

VARIETIES.

Of three kinds. (A) Arteries, (B) Veins, and (C) Capillaries.

(A) ARTERIES.

PREPARATION.

Longitudinal and *transverse* sections of a medium-sized artery (or vein), which has been hardened in a 1°/₀ solution of potassium bichromate, should be stained in logwood, prepared, and mounted in Canada balsam.

STRUCTURE.

Arteries (except those of minute size) have three coats:—

1. Internal coat, consisting of (*a*) *An epithelial layer*, forming the lining of the vessel, of thin elliptical or irregularly polygonal cells, often lanceolate, with nuclei and nucleoli; (*b*) *A subepithelial layer* of delicate connective tissue, with branched corpuscles; (*c*) *Elastic layers* of longitudinal elastic networks and "fenestrated" membrane. 2. Middle coat chiefly consists of circular *unstriped muscle fibres*, mixed with *elastic fibres*, and a sparse amount of connective tissue. 3. External coat (tunica adventitia) chiefly consists of fine and closely-felted bundles of *connective tissue*, together with longitudinal elastic tissue between them.

NOTE.—In the largest arteries, the middle coat consists of *alternate* layers of elastic tissue and unstriped muscle.

(B) VEINS.

PREPARATION.

As of the arteries.

STRUCTURE.

As of the arteries, with these differences:—

The elastic tissue of the internal coat seldom occurs in the form of fenestrated membranes. The middle coat is thinner, contains less muscular tissue and more white connective tissue. The external coat of some veins has a considerable amount of unstriped muscular fibres.

(C) CAPILLARIES.

PREPARATION.

Best obtained from the pia mater. The brain of some animal, such as a cat or dog, should be left for two days in a 2°/₀ solution of potassium bichromate, then the pia mater may be stripped off in pieces, stained, and mounted in the usual manner. Capillaries are well seen also in the mesentery of a cat or other animal.

STRUCTURE.

The walls of the capillaries proper are formed entirely of a simple epithelial layer of flattened lanceolate cells, joined edge to edge, and continuous with the layer which lines the arteries and veins. The larger capillaries have an outside structureless or finely fibrillated coat. In vessels rather larger (small arteries and veins) there is added, outside the epithelium, a thin layer of unstriped muscular fibre.

Tongue. 3 coats, 1. Mucous coat is covered with stratified epithelium & is provided with papillae (3 kinds in Human subject) Circumvallate fungiform & filiform & these again with small closely set secondary papillae, which are hidden under the epithelium, the secondary papillae are found everywhere in the mucous membrane & not over the larger papillae alone; but these latter are confined to special parts & as a rule are placed in circular depressions. Surrounding some of the papillae notably the Circumvallate are certain ovoidal or flask like bodies the so called "taste ~~tubes~~ body buds" composed of modified epithelium. Nerves are supposed to terminate in these cells. In the mucous membrane also are small tubular glands some secreting mucous but others whose ducts open into the trenches around the taste organs secreting a more watery fluid. Lymphoid tissue which here & there forms distinct follicular glands is found in large quantity in the mucous membrane at the posterior part. Crypts or recesses too are found, the walls of which are studded with nodules of lymphoid tissue.

2. The submucous coat is incomplete & scanty.

3. The muscular coat consists of longitudinal transverse & vertical bundles of striated muscles.

ALIMENTARY CANAL AND GLANDS CONNECTED WITH IT.—Paper II.

(C) TONSILS.

Preparation.

Sections made from a tonsil which has remained for a week in $\frac{1}{6}{}^{\circ}/_{0}$ solution of chromic acid, and subsequently in spirit, should be stained with logwood, and mounted in Canada balsam. An enlarged tonsil which has been removed from a child will answer the purpose.

Structure.

A tonsil consists of an elevation of the mucous membrane presenting upon its surface fifteen orifices leading into crypts or recesses, in the walls of which are placed nodules of lymphoid tissue. These nodules are enveloped in a less dense lymphoid or adenoid tissue which reaches to the mucous surface. The mucous surface is usually covered with squamous epithelium, and may present rudimentary papillæ which are then formed of adenoid tissue. The tonsil is bounded by a fibrous capsule. Into the crypts open a number of ducts of mucous glands.

(D) ŒSOPHAGUS.

Preparation.

Small pieces of the œsophagus of a dog both of upper and lower parts should be hardened in chromic acid and spirit. The mucous glands are best seen in sections from the lower part of œsophagus.

Structure.

Of three coats :—

1. An *external* or *muscular coat* consists of two layers, longitudinal and circular, the former or external layer at the commencement being disposed in three fasciculi, one in front and one on each side. At the upper end of the œsophagus, the muscular coat is red, and consists of striated muscle; lower down it becomes paler, and the fibres are mostly unstriated. 2. A *submucous coat* consists of areolar tissue, and contains mucous glands (tubular) whose ducts pass through to open on the mucous membrane. 3. A *mucous coat* which is firm and wrinkled, provided with minute papillæ, and covered with thick stratified scaly epithelium. It is separated from the submucous coat by a layer of unstriated longitudinal muscular fibres, which is partially imperfect as a layer above but complete below (*muscularis mucosæ*).

Salivary glands.

Capsule of fibrous tissue which sends septa into the substance of the gland supporting blood-vessels lymphatics, nerves & ganglia

Structure. as of compound tubular glands in which a lobule is made up of convolutions of a main division of a duct bound together with connective tissue. The convoluted parts are lined by & almost filled with a single layer of columnar cells (salivary cells) enclosing a nucleus. The granular appearance seen in salivary cells is due to dense network of fibrils which they contain. These cells when isolated are not unfrequently found to be branched. The basement membrane of the tubes consists of branched & flattened cells & between it & the salivary cells are found here & there (not in the parotid) granular semilunar bodies "the semilunes of Heidenhain". Mucous or goblet cells are also found in the lumen of the tubes in the mucous secreting gland. The smallest divisions of the ducts have a relatively small lumen & are lined near the convolutions with flattened epithelium & then with nucleated columnar cells which present a longitudinal striation. The larger ducts acquire an outside coating of connective tissue & are lined with a single layer of columnar epithelium containing an intracellular network of fibres arranged longitudinally. In the walls of the largest ducts are unstriped muscular fibres

NB
Compound racemose glands

ALIMENTARY CANAL AND GLANDS CONNECTED WITH IT.—Paper III.

Preparation.

Sections of hardened pieces of stomach, duodenum, ileum, and colon, made in various directions, should be stained in logwood; some of those of the stomach should be placed in 1°/₀ solution of anilin blue and left in it for half an hour, so as to stain thoroughly. After staining, prepare, and mount as usual.

(E) STOMACH.

Structure.

Of four coats:—

1. *Mucous or internal coat,* is smooth, soft and pulpy, pink, becoming gray soon after death. Thickest at pylorus, thinnest at the great curve. Loosely connected with the muscular coat by means of the submucous, and so presenting temporary ridges (rugæ) when the organ is contracted. It consists almost entirely of small tubular glands, arranged close to and parallel with each other, varying in diameter from $\frac{1}{500}$th to $\frac{1}{360}$th of an inch, and in length from $\frac{1}{60}$th to $\frac{1}{20}$th of an inch, lined to a variable extent by columnar epithelium, which also covers the whole of the mucous membrane. The tubular glands are for the most part simple, except near the pylorus, where they become larger, longer, and branched. The glands consist of a basement membrane formed of branched stellate cells joined edge to edge, and sending processes on the one hand to join the retiform tissue of the mucous membrane, and on the other to support the gland cells. The glands are of two kinds, differing chiefly in the character of the cells and their secretion. The one, or so-called "*mucous glands*," often branched, confined to the pylorus, are lined throughout by columnar epithelium, although towards the "fundus" or closed extremity of the gland, it tends to become cubical. The other, or "*peptic glands*," distributed throughout the stomach, except at pylorus, but best marked perhaps towards the cardia, less often branched, are lined to a variable extent by columnar epithelium, which is succeeded at the neck of the gland by large, coarsely granular cells ("peptic cells"); at the fundus these peptic cells do not form a regular lining to the gland, but occur here and there, producing bulgings of the basement membrane, the rest of the tube, except a small central channel (lumen) is filled with finely granular, polyhedral or angular cells ("central cells"), which are said to be continuous above with the columnar cells of the neck, and which also resemble the cubical cells lining the fundus of a mucous gland. Between and beneath the

mucous [illegible]
at pylor

peptic a
except pylo

glands is a quantity of delicate connective tissue, together with a small amount of retiform or lymphoid tissue, which here and there is collected into small masses somewhat resembling the solitary follicles of the intestine. A double layer (circular and longitudinal) of unstriated muscle (muscularis mucosæ) separates the mucous membrane from the submucous coat. 2. The *submucous coat* consists of areolar tissue with some fat, together with blood-vessels and lymphatics; small nerve ganglia and fibres are also found in it. 3. The *muscular coat* consists of three layers of unstriated fibres, externally of longitudinal, then of circular, and internally of oblique fibres; the circular layer is the only complete one. Between the layers may be found *plexuses of nerves.* 4. The *serous coat* is the peritoneal covering of the organ.

NOTE.—It may be as well to mention that the so-called "peptic cells" are no longer thought to secrete the pepsin.

ALIMENTARY CANAL AND GLANDS CONNECTED WITH IT.—Paper V.

(H) PANCREAS.

Preparation.

As of the salivary glands.

Structure.

The capsule and septa as well as the blood-vessels and lymphatics are arranged as in the salivary glands. It does not, however, contain the semilunar granular bodies ("semilunes of Heidenhain"), and it is looser and softer, the lobes and lobules being less compactly arranged.

The larger ducts possess a very distinct lumen, and a membrana propria lined with columnar epithelium cells which are longitudinally striated, but are shorter than those found in the ducts of the salivary glands. *In the smaller ducts* the epithelium is short and the lumen is smaller. The *intermediary ducts* opening into the alveoli possess a distinct lumen, with a membrana propria lined with a single layer of flattened elongated cells. *The alveoli* are branched and convoluted tubes, with a membrana propria and a single layer of columnar cells. The cells consist of an outer part nearest the membrana propria which is homogeneous and stains the more deeply; and an inner, more granular, and less readily stained portion. The alveoli have no distinct lumen, its place being taken by fusiform or branched cells.

(I) LIVER.

Preparation.

Small portions of the fresh liver of a pig, rabbit, or puppy, should be steeped for four or five days in a 2°/$_0$ solution of potassium bichromate, and then for one or two days in methylated spirit. Sections should then be cut and treated as usual. It is as well also to mount sections of liver which has been injected through the portal vein with 2°/$_0$ solution of Berlin blue, and then hardened in spirit.

TRUCTURE.

It has a serous and fibrous coat. The former is absent from the posterior border and from the portal fissure, where the latter, which elsewhere is thin, is most developed. A strong sheath of areolar tissue ("*Glisson's capsule*") surrounds the vessels of the organ as they ramify in it, and, at the transverse fissure, ecomes continuous with its fibrous coat. *The liver-substance oper* consists of lobules, which are closely packed polyhedral masses more or less distinct, arranged round the sides of the branches (sublobular) of the hepatic veins and connected to them by minute veins which begin in the centre of the lobules (intralobular veins). *Each lobule* consists of a mass of compressed spheroidal or polyhedral nucleated and nucleolated cells from $\frac{1}{1030}$th to $\frac{1}{830}$th of an inch in diameter, often containing oil globules. Surrounding the lobules is a variable amount of fine connective tissue in which is contained a minute branch (~~intra~~inter-lobular) of the portal vein, a branch of the hepatic artery and of the hepatic duct, together with minute lymphatic vessels covering them. The lobules are distinct when the intralobular tissue forms complete septa around them; if the septa are incomplete the lobules become confluent. Fine fibrous tissue surrounds the intralobular vein, and a delicate supporting network of flattened branched corpuscles exist within the lobule between the cells and the blood-capillaries. Between the columns of the cells run the radicals of the hepatic vein which open into the intralobular vein, and between the cells begin the radicals of the hepatic duct. Whether these radicals or bile capillaries have a definite membrana propria is undetermined. The ~~intra~~interlobular *bile ducts* are endothelial tubes with a large lumen, lined with columnar epithelium. The larger ducts are surrounded with circular unstriped muscle cells, and have a distinct mucous membrane of loose connective tissue lined with columnar epithelium, and containing mucous tubular glands. The lymphatics of the lobule originate in the spaces around the capillaries of the lobules.

GALL BLADDER

is similar in structure to the large hepatic ducts, but the *mucous membrane* is thicker, and is thrown into folds and villous projections. *The muscular coat* also is thicker, and is surrounded by *connective tissue*, and an outer layer of *peritoneum.*

TRACHEA AND LUNG.

PREPARATION.

Distend the lungs of a recently killed rabbit or cat, through the trachea, with $\frac{1}{6}\,{}^{\circ}/_{0}$ solution of chromic acid, tie up the trachea and immerse it in a large quantity of chromic acid of similar strength. Change the solution for one of a $\frac{1}{4}{}^{\circ}/_{0}$ in two days; in a week cut in pieces, and remove to methylated spirit.

Before cutting sections, it is necessary that the embedding mass shall have thoroughly penetrated into and filled up the interstices of the tissue, and so it is best to place the piece of lung to be embedded in the wax mass when it (*i.e.* the lung) is quite hot. In some cases it is as well to stain the lung with logwood, and pass it through alcohol and oil of cloves before embedding. Unless the interstices are filled up it is almost impossible to cut thin sections.

To free the cut sections from wax, pass them through oil of turpentine before putting them into oil of cloves.

Thin sections of lung injected through the pulmonary artery with Berlin blue, and through the trachea with $\frac{1}{2}{}^{\circ}/_{0}$ solution of silver nitrate, should be made if possible, and treated in usual manner. Sections of trachea should also be made.

STRUCTURE.

(1) *Of Trachea.* (*a*) *An elastic framework* of incomplete rings or hoops of hyaline cartilage, 16 to 20 in number; each presents a curve of rather more than $\frac{2}{3}$ of a circle. These rings are held together by a strong fibrous membrane, more or less elastic, which not only occupies the interval between them, but is prolonged over their outer and inner surfaces; behind, where the cartilage is incomplete, the fibrous membrane is strengthened by a continuous layer of unstriated muscle, principally transverse. (*b*) *A submucous coat* of areolar tissue and fat, containing also, immediately beneath the mucous membrane, longitudinal fibres of elastic tissue, collected for the most part into bundles. Mucous tubular glands are found in this coat, and also upon and beneath it. (*c*) *A mucous membrane* containing a large amount of lymphoid tissue, under the epithelium a basement membrane of flattened cells, which send up processes to the epithelium. In the deeper parts are many elastic fibres. On the surface are several layers of epithelium, of which the more superficial are columnar and ciliated, often branched below to join the connective tissue corpuscles. Between the branched ends of these cells are smaller elongated cells, prolonged upwards towards the surface, and down-

wards to the basement membrane. Beneath these are one or more layers of less regularly shaped cells. (2) *Of Bronchi* as of trachea. (3) *Of Lung.*—The tissue is made up of lobules attached to the minute divisions of the air-tubes by which they are held together, as well as by blood-vessels and interlobular tissue. The lobules, although adherent, are quite distinct ; the structure of each represents that of the entire lung, and consists of a minute *air-tube* with terminating *air-cells,* lined with tesselated *epithelium,* together with the pulmonary and bronchial blood-vessels, lymphatics, nerves and areolar tissue. The principal divisions of the bronchi divide, generally dichotomously, into branches running in all directions, which never anastomose, but terminate separately in the lobules ; within the lobule each bronchial tube finally ends in small recesses (air-cells, alveoli, or vesicles) having previously lost its cylindrical form, from being beset with similar air-vesicles on all sides ; in this condition the tube becomes what is called an *infundibulum.*

The structure of the air-tubes gradually changes as they become smaller. The cartilages become irregularly-shaped plates and rings of different sizes, scattered over the sides of the tubes, gradually becoming fewer, and finally disappearing before the infundibulum is reached. The fibrous coat extends to the smallest tubes, by degrees becoming simply areolar. The mucous membrane becomes thinner, but retains its former epithelium. The longitudinal elastic bundles are traceable into the smallest tubes. The muscular fibres ultimately form a continuous circular layer inside the cartilaginous plates. At the infundibulum the muscular fibres disappear, the elastic tissue becomes scanty, and the epithelium cubical. Finally, the walls of the air-vesicles are composed of connective tissue, elastic fibres, and a few muscular fibres, and are lined with tesselated epithelium. Under the epithelium is a dense capillary network.

SKIN AND APPENDAGES.—Paper I.

Preparation.

Small pieces of skin from various parts—*e.g.*, from palm of hand, fingers, or toes, scalp, scrotum, and general surface—should be hardened in equal parts of chromic acid, $\frac{1}{2}°/_{\circ}$, and of methylated spirit, for a week, changing the liquid on the 2nd, 4th, and 7th days, and then removing to spirit until required. Sections may be made in various directions (cutting towards the epidermis is the easiest way), stained, prepared, and mounted in the usual manner. Double staining, with picrocarmin as well as with logwood, is recommended. Injected specimens of skin may also be prepared by injecting $2°/_{\circ}$ Berlin blue solution into the main artery of a limb of a dog, or of one of the upper extremities of a new-born fœtus.

Structure.

The skin consists of two parts:

1. *Epidermis, or external skin*, is made up of several more or less distinct layers. The most superficial horny layer (stratum corneum) varies in thickness, is composed of layers of flattened epithelium, which show nuclei only after treatment with softening reagents, *e.g.*, caustic potash. (2) The next layer (stratum lucidum) is generally homogeneous and thin; it is composed of closely-packed scales. (3) A layer of granular cells (granular layer), flat, spindle-shaped, and nucleated, which stain deeply in logwood. (4) Finally the Malpighian layer (rete Malpighii, or rete mucosum), consisting of stratified epithelium, the deepest layers of which are columnar, the next more or less cubical "ridged" cells, connected together by filaments or prickles, and most superficially are layers of flattened cells.

2. *Internal, or true skin* (*corium or cutis vera*), is made up of dense areolar tissue, in which is found lying deeply a good deal of fat; muscular fibres occur in the neighbourhood of hairs, they exist as a distinct layer in the subcutaneous tissue of certain parts, *e.g.*, scrotum, penis, areola, etc. In the superficial part of the corium are numerous conical elevations or papillæ, which are received into corresponding pits in the epidermis; they are most developed where sensation is most acute. The subjacent or reticular part of corium contains hair follicles, with sebaceous glands, and also in subcutaneous tissue sweat glands (mostly simple tubular glands).

Nerves and blood-vessels are numerous: the former ending in the Malpighian layer in a delicate network, and supplying certain of the papillæ, form special endings (end bulbs and tactile corpuscles); the latter form near the surface a dense network of capillaries with rounded polygonal meshes.

lined & almost filled with granular epithelium. after becoming smaller it passes straight down the Pyramid towards the papillae & returns again forming "a looped tube of Henle" lined with squamous epithelium then again becom convoluted & finally joins a branch of a straight tube of the pyramid (Collecting tube) The Collecting tubes are lined with columnar epithelium & joining together form the excretory tubes which open at the papillae Blood is supplied to the Kydney by the Renal artery which divides into branches from which smaller vessels enter between the papillae & proceed in the cortex dividing & subdividing to reach the base of the pyramids where they form arches between cortical & medullary parts from which branches (~~interlobular~~ (interlobular) supply the afferent vessels of the Malpigian tufts; these break up within the Capsule into convoluted capillaries reuniting into the efferent veins, these again break up into capillaries round the convoluted tubes to be afterwards collected into small branches of the Renal veins Arterial vessels (vasa recta) are also found in the medullary part of the organ and corresponding veins. A certain amount of interstitial connective tissue is found supporting the tubes & blood vessels.

SKIN AND APPENDAGES.—Paper II.

HAIR.

Preparation.

May be seen in sections of skin or scalp; they may be examined in any reagent. Transverse sections are well made in the ordinary operation of shaving.

Structure.

The free extremities of hairs above the skin are pointed, the attached extremities are received into follicles in the corium; between the extremities is the shaft. The follicular end is bulbous and cased in a compound sheath.

A hair itself is made up of (*a*) an external covering of thin scales (cuticle); (*b*) a cortical substance made of coloured horny matter; and finally (*c*) the medulla or pith, which is absent in some hairs.

The bulb of the hair rests upon and overlies an elevation of the follicle (papilla), which is composed of undeveloped nucleated connective tissue corpuscles, and a few fibres. The sheath of the hair is divided into (*a*) internal, of two layers of large cells, the external layer consisting of transparent oval cells without nuclei, and the other layer of polyhedral nucleated cells; (*b*) external, of a variable number of layers of cells, becoming more columnar externally. The hair follicle consists of an involution of the cutis vera, forming three layers: (*a*) external is very thin, made up of longitudinally arranged connective tissue bundles, with fusiform nuclei and elastic fibres; (*b*) middle is thicker, and made up of transverse, undeveloped fibrous tissue, with rod-shaped nuclei; (*c*) internal, of a thin, striated, transparent membrane of endothelial cells. To the outside of the follicles thin bundles of unstriped muscular fibre are attached, and into the follicle open the ducts of sebaceous (simple tubular) glands generally one on each side.

NAILS.

Preparation.

Sections may be cut of the end of a finger after hardening.

Structure.

Nails are composed of flattened epithelial scales, and are equivalent to the superficial or horny layer of the epidermis. The deeper layers of the nail are softer than the more superficial. Underneath the nail are highly vascular papillæ (bed or matrix). Posteriorly it is received into a groove in the skin (root). The growth of the nail is effected by constant additions of cells to the root and under surface, so that it grows in length and in thickness at the same time.

Kidney.

Structure. There is a distinct fibro areolar coat, thin smooth & easily detached. The proper substance of the organ is divided into Cortical & medullary portions. Medullary, dense, deep red & distinctly striated is arranged in separate conical masses (pyramids) with their bases outwards & their points (papillae) towards a cavity in the interior of the organ (sinus) The Cortical substance, lighter in colour separates the pyramids from each other & encloses them every where except at the papillae; one layer of it situated immediately beneath the capsule forms the most superficial part of the organ. The papillae are studded with minute openings leading into tubes (tubuli uriniferi) through which the urine passes out into a primary division (infundibulum) of the pelvis or dilated part of the duct (ureter) of the kidney. The tubes of the pyramids as they pass out divide again & again at very acute angles until they arrive at the cortical layer & then become convoluted. The several bundles of tubes from each pyramid become conical as the central part becomes convoluted nearer the surface than the more external (pyramids of Ferrein) The tubes begin in spherical dilatation (Malpighian Capsules) enclosing tufts of minute vessels (Malpighian tufts) Arising thus in the cortex a tube is at first convoluted & consists of a basement membrane

GENITO-URINARY ORGANS.—Paper II.

(B) URETER.

Preparation.

Tie one end of the ureter, and distend it with chromic acid and spirit; leave for one day in the same mixture; then slit open and leave in spirit for a week. The cells may be shewn by hardening a piece of ureter in bichromate of potash 1°/₀ solution, staining deeply in logwood, and scraping the inside, teasing and mounting in glycerin.

Structure.

Consists of three coats:—(1) External fibrous; (2) middle of two layers (circular and longitudinal) of unstriped muscular fibres; (3) internal or mucous, lined by stratified epithelium, the upper cubical cells of which have their under surfaces hollowed out to receive the second layer of pear-shaped cells.

(C) BLADDER.

Preparation.

As of the ureter.

Structure.

Consists of three coats:—(1) Serous or external—is incomplete, as it is only found at the upper and posterior parts. (2) Muscular, consisting of three layers more or less complete—viz., (*a*) external longitudinal, (*b*) circular, (*c*) internal longitudinal. (3) Submucous of connective tissue. (4) Mucous, lined with stratified epithelium, the upper layer being made up of polyhedral cells, with one, two, or three nuclei, presenting depressions with intervening ridges for the second layer of club-shaped cells; the next layer is made up of more spindle-shaped cells.

(D) PROSTATE.

Preparation.

$\frac{1}{4}$°/₀ chromic acid for two days, followed by spirit.

Structure.

Small racemose glands embedded in an abundance of muscular fibres and connective tissue. The glandular substance consists of numerous small saccules, opening into elongated ducts, which unite into a smaller number of excretory ducts. The epithelium in the acini, as well as in the ducts, is columnar. The acini, in the upper part of the gland, are small and hemispherical; whilst in the middle and lower parts the tubes are longer and more convoluted. The tunica adventitia is formed of loose connective tissue containing fat. Large vessels pass into the interior of the organ to form a broad-meshed capillary system. Nerve trunks, and numerous large ganglion cells surround the cortex. Pacinian bodies are also found in the substance of the prostate.

(E) VAS DEFERENS.

PREPARATION.

By hardening in a 2°/₀ bichromate of potash solution for fourteen days, after which the tissue is to be placed in spirit.

STRUCTURE.

Like the vesiculæ seminales, of three coats—(1) External of connective tissue, outside which longitudinal fibres of unstriated muscle are often seen. (2) Muscular, two longitudinal layers with an intermediate circular one. (3) Mucous, of connective tissue, and elastic fibres ; this layer is often thrown into three or four longitudinal ridges; the epithelium consists of columnar epithelium, ciliated only near the epididymis. (4) The nerves form a plexus in the tunica adventitia.

GENITO-URINARY ORGANS.—PAPER III.

(F) TESTES.

PREPARATION.

Place the testes, preferably of rat or cat, after making two or three cuts in it, in equal parts chromic acid $\frac{1}{2}°/_{0}$ and methylated spirit. Change three times in a week and remove to spirit, or inject a $1°/_{0}$ solution osmic acid into the tunica albuginea, then place in strong spirit for several days, and afterwards for two days in absolute alcohol previous to making sections. Stain with hæmatoxylin or carmine; prepare and mount as usual.

STRUCTURE.

(1) Outer coat of connective tissue, the *tunica albuginea*, from which radiate incomplete septa uniting into a thick wedge-shaped body, the corpus highmori. The testis is divided by these septa into lobes each consisting of small and convoluted tubes, the tubuli seminiferi. (2) The *tubuli seminiferi* are composed of a basement membrane of flattened endothelial cells, a single row in small animals, more than one in large animals, within which are a number of cells not arranged in any definite order—the seminal cells. (3) From the *seminal cells* the spermatozoa are developed. The tubuli seminiferi have a uniform diameter of $\frac{1}{150}$—$\frac{1}{200}$ in.; they commence in free closed extremities or in anastomosing arches, and unite to form the vasa recta. In transverse section the seminal tubules are seen to have a narrow lumen surrounded by polygonal cells, of which the peripheral ones are arranged radially. In the interstitial connective tissue between the tubuli seminiferi are a number of connective-tissue corpuscles. (4) The *vasa recta*, about twenty in number, are $\frac{1}{90}$—$\frac{1}{70}$ in. in diameter. They possess very thin walls, and pass upwards and backwards to terminate in the rete vasculosum testis. (5) The *rete testis* is lined with pavement epithelium, and opens into twelve to twenty vasa efferentia, forming the coni vasculosi. (6) The *coni vasculosi* are $\frac{1}{50}$ in. in diameter, and open into the canal at the epididymis. (7) The *epididymis* and *vasa efferentia* contain plain muscular fibres; the lining cells are columnar and ciliated, elongated in the epididymis, shorter in the vasa efferentia. (8) Remove and draw *spermatozoa* from the fresh glands, (*a*) the head, (*b*) the middle portion, (*c*) the caudal extremity. (9) The *blood-vessels* surround the convoluted tubules with a long-meshed wide capillary plexus. (10) The *lymph passages* form an extensive canalicular system.

vasa recta
[illegible]
tubuli contort
seminal [illegible]
[illegible]

(G) VESICULÆ SEMINALES.

PREPARATION.

Either in $\frac{1}{6}°/_{0}$ chromic acid for seven days, followed by spirit, or the hardening may be effected by spirit alone.

STRUCTURE.

(1) External *connective tissue* coat. (2) Middle *muscular* coat of three layers, the internal of longitudinal fibres, middle of circular fibres, external of longitudinal fibres. (3) *Mucous* coat thrown into rugæ, the epithelium of cylindrical cells provided with striated borders. The mucous membrane contains a few muscular fibres. (4) *Ganglion cells* and *nerve plexuses* are numerous in the outer coat. (5) According to Leydig, a number of racemose glands are present.

GENITO-URINARY ORGANS.—Paper IV.

(H) OVARY.

Preparation.

The ovaries are placed, with as little handling as possible, in a mixture of equal parts of spirit, and $\frac{1}{2}{}^{\circ}/_{\circ}$ chromic acid solution for two or three days, and afterwards in spirit. The sections are to be stained with hæmatoxylin or carmine.

Structure.

The ovary consists of an encapsuled stroma, and embedded Graffian follicles. *The outer coat* consists of low columnar epithelium cells, beneath which is a firm layer of fibrous tissue. *The stroma* of fibrous tissue and elastic fibres, containing blood-vessels, and in the deeper portion, muscular fibres. The cortical portion contains a large number of closely-set vesicles, $\frac{1}{100}$ in. in diameter. Each vesicle, or primordial ovum, is surrounded by a corona of small nucleated cells. Below this layer of vesicles are more advanced ova, the deepest being the most mature. *The Graffian follicle*, $\frac{1}{20}$ — $\frac{1}{6}$ in. in diameter, contains a ripe ovum ; and is surrounded by fibrous tissue, and by the tunica vasculosa, more internally by the tunica granulosa, consisting of several layers of granular prismatic cells. In a thickened portion of the tunica granulosa (discus proligerus), the ovum is embedded, on the inner surface and to one side of the Graffian follicle. The tunica granulosa is separated from the discus proligerus, except at their point of union, by a space containing a clear albuminous fluid. *The ovum*, $\frac{1}{120}$ in. in diameter, consists (*a*) of an external, firm, transparent membrane, which is finely striated radially (vitelline membrane, or zona pellucida), (*b*) of a mass of granular protoplasm (vitellus, or yolk), (*c*) of a small clear vesicle, $\frac{1}{700}$ in. in diameter (germinal vesicle), embedded in the vitellus, and which encloses (*d*) a dark granular spot,—(germinal spot or macula germinativa), $\frac{1}{3000}$ in. *The corpus luteum* is a Graffian follicle which has discharged its ovum ; it is filled with a reddish-yellow mass of elongated cells, the colour being due to the formation of pigment, which, however, is not derived from the slight hæmorrhage which takes place on the escape of the ovum.

(I) UTERUS.

Preparation.

Distend with a mixture of equal parts of $\frac{1}{2}{}^{\circ}/_{\circ}$ chromic acid solution and strong spirit through vagina. Tie the openings into the organ, and remove to a bottle containing same mixture. Change solution at the end of twenty-four hours, and lay open the uterus. Two days later remove to spirit. Stain in hæmatoxylin.

STRUCTURE.

(1) *External serous coat* derived from the peritoneum. (2) *Muscular coat* intermixed with fibro-areolar tissue, blood-vessels, lymphatics, and some veins. The muscle is arranged in three layers: (*a*), the external longitudinal, the weakest coat; (*b*), transverse fibres forming the strongest layers; (*c*), oblique fibres which become annular to form the sphincter uteri. The cells constituting the muscular layers are fusiform, with long tapering extremities; the nucleus is always single. (3) *The mucous membrane* is smooth in the fundus and body of the organ; it is raised into transverse folds in the upper portion of the cervix; and forms papillæ in the terminal portion of the cervix. It is lined with columnar ciliated epithelium. The glands are tubular, often spiral, sometimes slightly branched. They are found in the fundus and body, and are lined with ciliated epithelium. Small closed sacs (ovula Nabothi), are also distributed regularly over the mucous membrane. (4) *The blood-vessels* are large and numerous; the *lymphatics* form large plexuses in the peripheral layers of the pregnant uterus; *the nerves* are medullated and non-medullated, a few ganglion cells being also present.

(J) FALLOPIAN TUBES.

PREPARATION.

As for the uterus.

STRUCTURE.

(1) *External serous coat*, rich in vessels and in connective tissue. (2) Longitudinal and a thicker circular layer of unstriated *muscle.* (3) *Mucous membrane* thrown into longitudinal rugæ, and lined with columnar ciliated epithelium; no glands are present, and as yet no nerves have been detected. The mucous membrane contains a layer of muscularis mucosæ.

THE LYMPHATIC SYSTEM.

I. LYMPHATIC VESSELS,

(A) TRUNKS, (B) CAPILLARIES,

are tubes with delicate transparent walls whose office is to convey the lymph and chyle. They arise by microscopic branches in nearly all the tissues of the body, and terminate by joining the great veins at the root of the neck. They are all efferent vessels.

(A).—PREPARATION.

Make sections of a thoracic duct which has been hardened in bichromate of potash and subsequently in spirit, stain with logwood, and mount in Canada balsam.

STRUCTURE.

Lymphatic trunks, such as the thoracic duct and the lymphatics leading to the mesenteric glands, have nearly the same structure as veins, and like them consist of three coats. They are provided with valves, especially at their subdivisions. The endothelial cells lining them are elongated.

(B).—PREPARATION.

To demonstrate the structure of lymphatic capillaries, the epithelium covering the central tendon of the diaphragm of a rabbit or guinea-pig must be pencilled off with a camel's-hair brush, stained with nitrate of silver, and mounted in glycerin.

STRUCTURE.

Lymphatic capillaries consist of a single layer of sinuous endothelial cells, united together by intercellular substance so as to form a membrane.

II. LYMPHATIC GLANDS

are nodal accumulations of adenoid tissue in the course of lymphatic vessels. They are round or oval in shape, and consist of a cortical and of a medullary part. Each receives lymphatic vessels called *vasa afferentia*, and from it other lymphatic vessels called *vasa efferentia* take their origin.

PREPARATION.

Thin sections of a lymphatic gland which has been previously hardened in Müller's fluid, or in bichromate of potash, must be stained with logwood, and shaken in a test tube half full of water for thirty minutes or more. They are then to be mounted in balsam in the ordinary manner.

STRUCTURE.

Each lymphatic gland is surrounded by a *capsule*, which consists of connective tissue intermingled with unstriped muscular fibres. From the capsule are given off a number of *trabeculæ*, which give support to the blood-vessels, and pass into the interior of the gland, so as to divide it into a number of compartments or alveoli, which contain the adenoid tissue or proper tissue of the gland. The *adenoid tissue* is arranged in the form of follicles in the cortex, and of rounded cords in the medulla. Between the walls of the alveoli and the proper tissue of the gland are a number of spaces lined by endothelium—the so-called *sinuses* of a lymphatic gland. These sinuses are continuous on the one hand with the afferent vessels, and on the other with the efferent vessels.

III. THE SPLEEN.

PREPARATION.

Small pieces of fresh spleen are hardened in 2°/₀ solution of bichromate of potash, and subsequently in spirit, till they are fit for making sections.

STRUCTURE.

The spleen possesses two coats, a serous and a fibrous. The *serous coat* is derived from the peritoneum, and covers the organ almost completely. The *fibrous coat*, or tunica propria, is composed of connective tissue which. in some animals, is intermingled with a large proportion of unstriped muscular fibres. From its inner surface processes or trabeculæ pass into the interior of the organ, and interlace freely with each other, so as to form the *trabecular framework* of the spleen. At the hilus of the spleen the capsule is prolonged along with the blood-vessels, for which it forms sheaths, which become connected with the trabeculæ above described. The interstices between these trabeculæ contain the proper tissue of the spleen, or *spleen pulp*, which consists chiefly of adenoid tissue. Small arteries pass off almost at right angles from the branches within the trabeculæ, into the spleen pulp, and exchange their outside coat of connective tissue for one of adenoid tissue. The spleen pulp contains in addition nodules of adenoid tissue, called *Malpighian bodies*, and red blood-corpuscles in all stages of development and decay. The Malpighian bodies appear to be cylindrical masses of adenoid tissue which pass through the whole organ; whether they have any necessary connection with the blood-vessels is uncertain.

THE EYE.—PAPER I.

CORNEA.

PREPARATION.

The anterior part of a pig's eye should be hardened in 2°/₀ solution of bichromate of potash for a fortnight, and then in spirit ; transverse and vertical sections should be made. To demonstrate the connective tissue cells and nerves, the fresh cornea of a frog or rabbit should be cut off, and put for about an hour into chloride of gold solution ½°/₀, then into slightly acidulated water and exposed to the light, and mounted whole (or in section in the latter case), in glycerin. In order to stain the cell spaces, the fresh or living cornea of a pithed frog should be pencilled with solid nitrate of silver, stained and mounted in glycerin.

STRUCTURE.

Consists of five layers :—(1) The superficial of stratified *epithelium*, of which the lower layer is columnar. (2) A thin homogeneous layer, the *anterior elastic lamina*, which does not seem to differ from the substance of the cornea proper, except in its greater density and the absence of corneal corpuscles. (3) The *proper substance* of the cornea, which is made up of alternating layers of fibrous tissue parallel to the surface. These layers are separated from one another by ground substance, in which are the cell spaces of irregular branched form which freely communicate with the cell spaces of the same, as well as of other layers. In the spaces, but not filling them up entirely, are the cornea corpuscles, branched cells of various forms. (4) Membrane of Descemet or *posterior elastic lamina*,—a firm, structureless, but brittle, transparent membrane, covered by a (5) layer of *epithelial cells.* There are no blood-vessels in a healthy cornea, except at the periphery. *Nerves* enter the proper substance of the cornea, becoming transparent form a plexus, from which finer branches going forward form another "sub-epithelial plexus," from which, again, finer fibrils pass among epithelial cells forming the "intra-epithelial plexus."

THE EYE.—PAPER II.

RETINA.

PREPARATION.

The posterior part of eye of pig (if no fresh human eye can be had), is hardened in Müller's fluid for a week, and then transferred to alcohol; pieces of the retina may then be stained in alcoholic logwood, and cut. Another method is to place in 2°/ₒ solution of osmic acid for four hours, then in water for one hour to get rid of the excess of osmic acid, and, finally, in alcoholic logwood. The retina, thus treated, will have to be embedded in cacao-butter, instead of the ordinary wax.

STRUCTURE.

Consists of eight layers in the following order, from within outwards:—

1. *Layer of nerve fibres*, which is wanting at the yellow spot: the fibres consist of axis cylinders only; it diminishes in thickness anteriorly.

2. *Layer of nerve cells* (ganglionic layer), consisting of cells of a spheroidal or pyriform figure; one process of each extends into the first layer, and is doubtless continuous with it. From the other end of the cell, one or more processes extend outward for a variable distance into the next layer. In the yellow spot there are several layers of cells; elsewhere, only one layer.

3. *Inner molecular layer*, a thick stratum of granular-looking substance.

4. *Inner nuclear layer* consists of transparent nucleus-like bodies, of at least four kinds—(*a*) a few connected with the fibres of Müller (to be described below); (*b*) the largest number, like bipolar cells, one pole unbranched, going inwards, and being connected with a nerve fibre,—the other, thicker and branched, running outwards, is supposed to break up into a plexus in the outer molecular layer; (*c*) unbranched cells found as a complete stratum at innermost part; (*d*) are scattered in the outermost part, are rounded, of large size, and have only one process.

5. *Outer molecular layer,* thinner than (3), but otherwise the same.

6. *Outer nuclear layer*, consists roughly of two kinds of corpuscles—(*a*) connected with the rods are most numerous, and may be considered as dilatations midway in the fine rod fibres, have an elliptical striped nucleus, and no nucleolus; (*b*) connected with the cones are fewer, pear-shaped, not striated, and nearer the outer part of the layer in the thicker cone-fibre.

7. *Layer of rods and cones*, is composed of elliptical elongated

bodies, *the rods*, and shorter, thicker, club-like bodies, *the cones;* each consists of two parts, inner and outer, of which the outer is transversely striated and smaller, and in the cones tapers to a point, whilst the inner is fibrillated externally, but homogeneous internally.

8. *Pigmentary layer*, consists of a single stratum of hexagonal pigment cells.

Fibres of Müller, consist of bands, which pass all through the layers of the retina, binding them together; they commence by a broad base, forming by their union the membrana limitans interna; and, at the outside of the retina, the membrana limitans externa. In the inner nuclear layer they give off processes, which contain a clear, oval, and elliptical nucleus. In the outer nuclear layer they break up into fibrils, and partially enclose the rod and cone fibres.

THE EAR.

COCHLEA.

PREPARATION.

The guinea-pig is best used. The animal being freshly killed, the lower jaw is disarticulated; by this means the tympanic bulla is exposed. The bulla is isolated, cleaned of its soft parts, and broken open, when the projection of the cochlea will be observed within it. The cochlea is to be still further isolated, by clipping away the surrounding bone; it is then placed in Müller's fluid for seven to fourteen days, and afterwards in a saturated solution of picric acid, until the bone is sufficiently softened; finally in weak spirit, followed by immersion in strong spirit. Stain in carmine and hæmatoxylin. (Schäfer.)

STRUCTURE.

The cochlea is a gradually-tapering spiral tube, winding round a central column, *the modiolus*. It is divided along its whole extent by a spiral lamina, which projects from the modiolus, into two main portions—the scala tympani, and the scala vestibuli. *The spiral lamina* is partially osseous, and partially membranous. The membranous portion, *the basilar membrane*, is connected to the outer wall of the cochlea by its union with the *spiral ligament*, which is a projection inwards of the periosteum and subperiosteal tissue of the cochlea. *The scala vestibuli* is subdivided into scala vestibuli proper, and *ductus cochleæ*, by the *membrane of Reissner*, which passes from the spiral lamina to join the lining periosteum. *The membrane of Reissner* is composed of a delicate layer of homogeneous connective tissue, continuous with the periosteum, covering the scala vestibuli. It is lined with a layer of flattened endothelium on the face turned towards the scala vestibuli; whilst that bounding the ductus cochleæ is provided with a single layer of squamous epithelium. *The periosteum* consists of ordinary connective tissue, thickened here and there by retiform tissue. *The spiral ligament*, to which the basilar membrane is attached, consists of periosteum thickened by the retiform tissue, the cells being elongated, and radiating from the attachment of the basilar membrane. At this point there is generally a large blood-vessel; whilst between the spiral ligament and the membrane of Reissner the periosteum contains pigment cells and a number of blood-vessels. *The floor of the ductus cochleæ* is formed of a narrow portion of the spiral lamina, and of the basilar membrane. This portion terminates in a border, which is "C"-shaped when seen in section, the lower limb of the "C" being prolonged and tapering. This limb is the end of the osseous lamina; it is covered by a thin membrane. The upper portion of the "C" is the *limbus* of the spiral lamina, whilst the bay of the "C" is called the *spiral groove*. *The limbus* has a jagged edge, as it is raised into a number of tooth-like projections. *The organ of Corti* forms a portion of the epithelium covering the basilar membrane; it consists of an outer and inner set of stiff rod-like bodies. The feet of the rods rest upon the basilar membrane, whilst they incline towards each other until they meet by their heads. By the meeting of the rods an arch is formed over the basilar membrane; it is filled with endolymph. On the inner side of the inner rod, and the outer side of the outer rods, are epithelial cells with short hair-like prolongations, the *inner and outer hair cells;* the outer cells are more numerous and more elongated than the inner cells. The hair-like prolongations of the outer hair cells project through rings which surround the tops of the cells, and which are bounded by minute, fiddle-shaped cuticular structures—*the phalanges. A reticular membrane* is thus formed which covers this part of the organ of Corti. On either side of the two sets of hair cells, the epithelium passes continuously into the simple layer of cubical cells, which is found in the spiral groove, and covering the outermost part of the basilar membrane. The whole organ of Corti is also covered by a thick and highly elastic *tectorial membrane. The inner rods* are smaller and more numerous than the outer rods ; they may be compared to the upper portion of the human ulna ; whilst *the outer rods* resemble the head and neck of a swan. The concavity of the inner rod receives the rounded portion of the outer rod, which would correspond to the back of a swan's head ; whilst the beak of the swan becomes connected to the reticular lamina. Both rods are more slender towards their middle, and expand again, so as to rest by a widened foot upon the basilar membrane ; both are longitudinally striated. In the head of the outer rod—and occasionally, also, in the inner rod—is an oval nucleus, staining more deeply than the rest of the cell. (Schäfer.)

Structure of the wall of the membranous semicircular canals. The wall consists, from without inwards, of (*a*) *an external fibrous layer*, containing numerous nuclei, blood-vessels, and irregular pigment cells. This layer is especially developed at the ends of the oval section where it coalesces with the ligamenta labyrinthi canaliculorum. (*b*) *The Tunica propria*, which presents, after staining, a delicately striated granular appearance. (*c*) *Capilliform processes*, which project into the interior of the canal, except at the part where the membranous canal touches the bone. (*d*) *The Epithelium*, a single layer of pavement epithelium cells investing the papillæ, and also continues into the depressions between them.

In the Ampullæ (*a*) *the fibrous layer* forms a loose meshed tissue, whilst (*b*) the *tunica propria* is so much thickened as to cause a rounded transverse projection into the cavity—*the crista acustica*, or *septum transversum.* (*c*) *The Epithelium*, covering the crista acustica, consists (i) of long cylindrical cells, each with a large nucleus ; these cells support the other nervous and epithelial elements, and rest upon the tunica propria ; (ii) fusiform cells which lie between the columnar cells : each cell has a long stiff cilium, *the auditory hair*, and is in direct connection with the ultimate fibrillæ of the auditory nerve. (*d*) *The Nerves*, after passing through the tunica propria, form a very delicate plexus in the epithelial layer.

THE TEETH.

PREPARATION.

(*a*) By means of sections of the hard tooth : Grind down the tooth on both sides till it is quite thin, then mount in hard Canada balsam, so as to retain the air in the various cavities. (*b*) By means of sections of the softened tooth : Place the tooth in $10^{\circ}/_{\circ}$ solution of hydrochloric acid till it is quite soft, then immerse in spirit; by this means the structure of the dentinal substances may be investigated ; or place the tooth (preferably broken across) in a saturated solution of picric acid until quite soft. Complete the hardening in spirit, changing the spirit as long as it becomes tinged with the picric acid. This method of preparation will preserve the pulp and odontoblasts. To demonstrate the pulp, break a freshly-extracted tooth, and immerse in osmic acid $\frac{1}{5}{}^{\circ}/_{\circ}$ for twenty-four hours. To show the development of teeth, place the lower jaw, cleaned of the muscles, of fœtal rat, dog, or kitten, in $\frac{1}{6}{}^{\circ}/_{\circ}$ chromic acid for seven days, then remove to weak spirit for twenty-four hours, and finally to strong spirit till required. (*c*) The dentinal sheaths lining the tubules may be isolated by boiling for ten minutes in strong sulphuric acid. In each case stain in hæmatoxylin.

STRUCTURE.

(1) A tooth consists most externally, and at the surface, of *enamel.* In the recently cut tooth, which has not been used, there exists above the enamel, a covering of epithelial or horny nature (Nasmyth's membrane), which is structureless, and has a thickness of $\frac{1}{30000}$ — $\frac{1}{15000}$ in. The enamel covers the crown and neck of the tooth ; it is an epithelial product, consisting of closely aggregated polyhedral cylinders (prisms or columns). The enamel fibres are crossed by a number of darker lines, arranged in concentric layers, "contour lines." In transverse section, the enamel fibres are seen to be six-sided prisms, with an average diameter of $\frac{1}{5000}$ in. (2) *The Dentine* covers the body and root of the tooth ; on the surface it lies immediately below the enamel. The dentine consists of a compact, bone-like substance, which contains no bone-corpuscles, and is permeated by dichotomously dividing canals, the dentinal tubules, which average $\frac{1}{4500}$ in. in diameter. The dentinal tubules run perpendicularly to the surface of the pulp cavity, into which they open by their lower extremities. The tubules present a proper wall, consisting of a membranous tube, and each contains a process of protoplasm from the superficial layer of the pulp cells. Examined under a low power, the tubules are seen to form two

or three gentle curvatures, which give rise, when a number of tubules are seen together, to a series of concentric lines (lines of Schreger). Certain interglobular spaces, due to imperfect deposition of salts, are also frequently seen in the dentine. (4) *The crusta petrosa*, or cement, invests the portions of the tooth which are not protected by enamel. It closely resembles bone in its histological appearances, except that the lacunæ and canaliculi are larger and more irregular. When the cement is very thick, it may contain vascular channels, which are comparable with Haversian canals. The perforating fibres of Sharpey are present in considerable numbers in the ivory. (5) *The pulp* occupies the central cavity of the tooth; it consists of jelly-like connective tissue, in which run nerves and blood-vessels. The outermost layer of cells, forming the pulp, are elongated in form, the bodies somewhat resembling columnar epithelium cells. This layer forms the membrana eboris; each cell is an odontoblast. The odontoblasts send off one or more processes, which run in the dentinal tubules; processes which connect the cells together laterally, and processes which unite the cells to others lying more deeply. (6) *Osteodentine*, or secondary dentine, is the hard substance deposited on the inner surface of the dentine, which is produced by the gradual corrosion of the pulp.

ERUPTION OF MILK TEETH.

Central incisors of	lower jaw	7th month.
,, ,,	upper ,, ...	
Lateral ,,	lower ,, ...	9th ,,
,, ,,	upper ,, ...	
First molar of	,, ,, ...	12th ,,
,, ,,	lower ,, ...	
Canines		18th ,,
Second molar		24th ,,

ERUPTION OF PERMANENT TEETH.

First molar	6 years.
Central incisors	7 ,,
Lateral ,, ...	8 ,,
Anterior bicuspids	9 ,,
Posterior ,, ...	10 ,,
Canines....................................	11—12 ,,
Second molars.............................	12—13 ,,
Third molars = wisdom teeth	17—25 ,,

For the Development of the Teeth see Appendix, pp. 110—11.

THE NOSE.

Preparation.

Small pieces of the upper turbinal bones from the head of a freshly killed sheep, dog, or rabbit, should be hardened in $\frac{1}{6}$°/₀ chromic acid solution for a week, and in $\frac{1}{4}$°/₀ bichromate of potash, or in 1°/₀ osmic acid, for forty-eight hours. Sections may be made through the nasal region of a newt or young guinea-pig's head which has been previously hardened in chromic acid and spirit. The fresh tissue may also be treated with chloride of gold.

Structure.

In a vertical section through the septum nasi, the osseous portion is seen to be invested by *periosteum*, which is immediately covered by a thick layer of numerous and elongated *tubular glands*, some simple, others more complex,—the glands of Bowman. These glands contain an epithelium of granular spherical cells at the base; of a more polygonal and less granular form near the excretory duct. The ducts open on the surface between the elements of the next external layer. The glands become less numerous and ultimately disappear at the point where the olfactory region passes into the ordinary mucous membrane, being replaced by the *mucous glands.* The glands are separated by ordinary *connective tissue*, in the deeper layers of which are *pigment cells* and free *pigment masses,* as well as *capillaries* and ramifications of the olfactory nerves. *The epithelium* is superficial, and consists of an external finely striated portion, and an internal granular layer. In the newt the epithelial cells are separated into groups, by teasing after maceration for forty-eight hours in Müller's fluid. Each group of cells consists of two kinds of cells, of which one is larger than the other, presents an elongated oval form, and is situated externally. *The olfactory cells*, the smaller of the two kinds, possess a large round nucleus, and two very long fine processes, of which the thicker runs outwards, whilst the finer is directed inwards. The external process is composed of two substances, an outer which swells up under the influence of certain reagents, and an internal thread which remains unaffected. In man and mammalia generally the olfactory cells have no cilia. The olfactory cells surround *the larger cells* provided with an oval nucleus which extend through the whole thickness of the epithelial layer. The external portion of these cells is more or less cylindrical, and is striated longitudinally. A row of dots can be distinguished upon the external extremities. The trunks of the *olfactory nerve* run in the glandular layer either obliquely or horizontally. The ultimate fibrils of the nerve pass into the epithelial layer and probably into the olfactory cells.

MAMMARY GLAND.

PREPARATION.

The gland in pieces is placed in a solution of equal parts of spirit and $\frac{1}{2}{}^{\circ}/_{o}$ chromic acid solution for two days, afterwards in weak and strong spirit. It is to be stained in hæmatoxylin.

STRUCTURE.

The mammary gland consists of a number of individual racemose glands united by intervening areolar tissue. (1) *The lobes* thus formed have a considerable quantity of adipose tissue between them, whilst the blood-vessels and the small medullated nerves run in the connective tissue stroma. (2) The racemose glands open by means of ducts, *the lactiferous ducts,* which unite together until 15 to 20 excretory canals are formed, (3) *the galactophorous ducts*, which converge towards the nipple. Near the nipple the galactophorous ducts become dilated to form sinuses, but they undergo constriction again before opening to the exterior. (4) *The gland vesicles* consist of a membrana propria with flattened stellate cells, lined by low columnar epithelium. The vesicles are filled with fat globules ; and if the oil be extracted by immersion of the gland in ether, casein remains behind. The terminal vesicles are at first simple, but as the gland developes they produce buds. (5) *The ducts* consist of areolar tissue with a circular and longitudinal layer of elastic fibres ; they are lined with low cylindrical epithelium, which becomes flattened near the nipple. Near the nipple also, and beneath the areola, unstriated muscular fibres are found.

DUCTLESS GLANDS.

THYROID GLAND.

PREPARATION.

Immersion of the gland for 24 hours in a mixture of spirit and water, then in strong spirit, till the tissue is sufficiently hard. The thyroid gland may also be hardened by allowing it to remain for a month in Müller's fluid, or in $\frac{1}{4}$°/$_{0}$ chromic acid for a fortnight. Stain in logwood.

STRUCTURE.

(1) A thin transparent layer of dense areolar tissue, free from fat, containing elastic fibres. This *connective tissue framework* traverses the interior of the organ in the form of strong trabeculæ; it encloses (2) rounded or oblong irregular cavities, *the vesicles.* The vesicles consist of a thin hyaline membrane lined by a single row of low cylindrical cells. The cavities of the vesicles are filled with a coagulable fluid, or more frequently with a colloidal substance. The colloidal substance increases with age, and the cavities appear to coalesce. (3) In the interstitial connective tissue is a round meshed *capillary plexus*, and (4) a large number of *lymphatics.* (5) *The nerves* adhere closely to the vessels.

THYMUS GLAND.

PREPARATION.

As for the thyroid gland, the concentric corpuscles may be teased out from the fresh gland in normal saline solution. Stain with carmine.

STRUCTURE.

(1) *A capsule* of thin areolar tissue which sends down processes dividing the gland into lobules. The outer surface of the organ is covered with a layer of flattened cells. (2) Each *lobe* is made up of a number of polyhedral lobules, connected by delicate areolar tissue, which are (3) in turn composed of small *follicles.* The follicles are composed of adenoid tissue, or retiform tissue the meshes of which are filled up with lymphoid corpuscles. The follicles are, therefore, comparable with the spleen, tonsils, lymphatic glands, and Peyer's patches. (4) Scattered in the adenoid tissue are the concentric *corpuscles of Hassall*, composed of deeply staining substance, with high refractive index. Of these there are two kinds—one, the smaller, simple; the other, the larger, compound. (5) *The arteries* radiate from the centre of the gland. (6) *The lymphatics* are large. (7) *The nerves* are very minute.

PITUITARY BODY.

PREPARATION.

As for the thyroid gland.

STRUCTURE.

Of two lobes—*a small posterior one*, consisting of nervous tissue; *an anterior larger one*, resembling the thyroid in structure. *A canal* lined with flattened or with ciliated epithelium, passes through the anterior lobe; it is connected with the infundibulum. *The gland spaces* are oval, nearly round at the periphery, spherical towards the centre of the organ; they are filled with granules and nucleated cells. The vesicles are enclosed by connective tissue, rich in capillaries.

PINEAL GLAND.

PREPARATION.

By hardening in alcohol, and by maceration in Müller's fluid; or, better still, by means of osmic acid.

STRUCTURE.

A central cavity lined with ciliated epithelium. The glandular substance is divisible into—(1) *An outer cortical layer*, analogous in structure to the pituitary body; and (2) *An inner central layer*, wholly nervous. The cortical layer consists of a number of closed follicles, containing (*a*) cells of variable shape, rounded, elongated, or stellate; (*b*) fusiform cells. There is also present a gritty matter, the acervulus cerebri, consisting of round particles aggregated into small masses. The central substance consists of white and grey matter. (3) *The blood-vessels* are small, and form a very delicate capillary plexus.

SUPRA-RENAL CAPSULE.

PREPARATION.

In bichromate of potash 2°/₀ for a fortnight, in Müller's fluid for a month, or in osmic acid four hours; in each case complete the hardening in spirit.

STRUCTURE.

(1) An outer *sheath of connective tissue*, which sends in prolongations, forming the framework of the gland. (2) *The cortical portion*, divided into (*a*) an external layer of closed vesicles, the zona glomerulosa. The vesicles contain a finely granular greyish substance, no fat globules, but generally a few small cells. (*b*) A layer of cells arranged radially, the zona fasciculata. The substance of this layer is broken up into cylinders, each of which is surrounded by the connective tissue framework. The cylinders thus produced are of three kinds—one containing an opaque, resistant, highly refracting mass (probably of a fatty nature); frequently a large number of nuclei are present; the individual cells can only be made out with difficulty. The second variety of cylinders is of a brownish colour, containing finely granular cells, in which are fat globules. The third variety consists of grey cylinders, containing a number of cells whose nuclei are filled with a large number of fat granules. (*c*) The third layer of the cortical portion is the zona reticularis. This layer is apparently formed by the breaking up of the cylinders, the elements being dispersed and isolated. The cells are finely granular, and have no deposit of fat in their interior; but in some specimens fat may be present, as well as certain large yellow granules, which may be called pigment granules. (3) *The medullary substance* consists of closed vesicles; of elements of the cortical substance; of numerous blood-vessels; and of an abundance of nervous elements. The cells are poor in fat, and occasionally branched, the nerves run through the cortical substance, and anastomose over the medullary portion.

PART II.

PHYSIOLOGICAL CHEMISTRY.

APPARATUS AND REAGENTS REQUIRED :

Test tubes.
Test tube stand.
Retort stand.
Platinum foil and wire.
3 Berlin dishes.
3 beakers.
Sand bath.
Filter papers and funnels.
Sulphuric, Nitric, Hydrochloric, Acetic, Tannic, Carbolic, and Boracic acids.
Chloride and sulphate of magnesium.
Sulphate of copper, 5°/₀ solution.
Carbonate of sodium.
Caustic potash, or soda.
Ammonia.
Millon's reagent.
Ferrocyanide of potassium.
Potassic and mercuric iodide.
Mercuric nitrate and chloride, mercurous nitrate.
Lead acetate.
Calcium chloride.
Alcohol.
Ether.

MEMORANDA OF EXPERIMENTS.

PROTEIDS are thus classified : —

I.—NATIVE ALBUMINS. Egg Albumin. Serum Albumin.

II.—DERIVED ALBUMINS. Acid Albumin. Alkali Albumin. Casein.

III. — GLOBULINS. Globulin. Fibrinoplastic Globulin. Fibrinogen. Myosin. Vitellin.

IV.—FIBRIN.

V.—PEPTONES.

COMPOSITION OF PROTEIDS :

C	H	O	N	S
52·7	6·9	20·9	15·4	0·8
to	to	to	to	to
54·5	7·3	23·5	16·5	2·0

Exist generally in a soluble and insoluble form.

General reactions :—

(i) *Turn yellow on heating with* HNO_3*; colour deepens on addition of ammonia* (xanthoproteic reaction).

(ii) *With Millon's reagent, a pink precipitate or mere coloration, either directly or on boiling.*

(iii) *With* $CuSO_4$ *and* $NaHO$ *a violet colour.*

(iv) *With acetic acid and ferrocyanide of potassium, a precipitate.*

(v) *Add acetic acid in excess, then* Na_2SO_4*: boil, and a precipitate will be formed.*

All act on polarised light.

CLASS I.—NATIVE ALBUMINS.

EGG ALBUMIN.

PREPARATION.

To white of egg in a test tube add about three or four times its volume of water, shake up and filter. Use solution for following experiments :

Evaporate to dryness at 40°C.

(Dry Albumin ; a yellow, transparent, glassy mass, soluble in water.)

Coagulation on simply boiling strong solution.

Precipitate on addition of strong acids (HNO_3, HCl,H_2SO_4).

No precipitate with organic acids except Tannic or Carbolic.

Precipitation by mercuric chloride, lead acetate, copper sulphate and silver nitrate without coagulation.

Coagulation by ether.

Coagulated albumin dissolved by caustic alkali.

TESTS FOR ALBUMIN.

Albumin precipitated from dilute solution by adding acetic acid and ferrocyanide of potassium.

Pour a little strong HNO_3 *into a test tube, and add gradually dilute solution of albumin*: albumin precipitated at point of contact with the acid, in the form of a fine ring.

SERUM ALBUMIN.

PREPARATION.

Dilute blood serum with water, add ether, shake up, filter and test filtrate with HNO_3.

Differs from egg albumin in not being coagulated by ether.

Also differs from egg albumin in being easily precipitated by HCl, and in being easily soluble in excess of this acid.

Serum Albumin, either in the coagulated or precipitated form, more soluble in excess of strong acid than Egg Albumin.

All albumin is very slightly diffusible.

EXPERIMENT.

Solution of albumin, to which salt has been added, should be placed on a dialyser, and the outside liquid tested for albumin, phosphates, and chlorides.

CLASS II.—DERIVED ALBUMINS.

Insoluble in water and in solutions of NaCl, but are soluble in dilute acids and alkalies.

ACID ALBUMIN.

If a small amount of dilute HCl or acetic acid be added to either egg or serum albumin, there is no precipitate or coagulation on heating.

On exactly neutralising the solution, a flocculent precipitate is produced.

The albumin has become insoluble in water and neutral saline solutions.

It is soluble in acids and alkalies. Insoluble in NaCl solutions.

All Globulins are converted by dilute acids into acid albumin.

Partial coagulation on boiling in lime water, and further precipitation on addition to boiled solution of $CaCl_2$,$MgSO_4$, or NaCl.

ALKALI ALBUMIN.

If solutions of albumin be treated with dilute alkali (NaHO), coagulation is prevented.

Alkali albumin thrown down on neutralising solution, except in the presence of alkaline phosphates.

To differentiate between Acid and Alkali Albumin the following method is useful :—

(1) Alkali albumin is *not* precipitated on exact neutralisation if sodium phosphate has been previously added.

(2) Acid albumin is precipitated on exact neutralisation, whether or not sodium phosphate has been previously added.

CASEIN.

Similar to alkali albumin, but differs in (1) yielding sulphide when heated with KHO to 110°; (2) yielding phosphorus-containing body when digested with gastric juice.

Obtained from milk by following process :—

Dilute the milk with three to four times its volume of water, add slight excess of dilute acetic acid, and filter off the casein.

To purify it, wash with alcohol and ether. Casein may also be prepared by adding to milk an excess of crystallized sulphate of magnesium, which causes it to separate out.

Casein is slightly soluble in dilute caustic alkalies and acids.

It is retained to a considerable extent in solution, by the presence of alkaline phosphates.

MILK.

EXAMINATION OF MILK.

Examine a drop of milk under the microscope with high power. See that it consists of fat globules of different sizes in a clear fluid. Add dilute acetic acid by irrigation, and observe the coalescence of the globules owing to the acid dissolving their casein membrane. Test the alkaline reaction of fresh milk; it becomes acid on standing, owing to the formation of lactic acid, whilst the casein separates.

The constituents whose presence is to be demonstrated are *Oil* or *Fat*, *Casein*, two kinds of *Albumin*, and *Lactose* or Milk Sugar.

FAT.

To a portion of milk add its own volume of KHO, and warm the solution *gently;* the casein will be dissolved from the oil globules. Add ether, shake the mixture and allow it to stand. The fat will be dissolved in the ether, and will form with it a clear superstratum. Remove the transparent top layer with a pipette, evaporate off the ether, the oil will be left, and will give the *characteristic greasy spot when dropped upon paper.*

CASEIN.

Dilute some of the milk with its own bulk of water; add a little dilute acetic acid until a slight granular precipitate is formed. Warm the solution *gently*, and a copious flocculent precipitate will fall. Filter off, and label the precipitate A.

ALBUMIN.

Boil the clear filtrate (from A), a coagulum of albumin will be formed. Filter this off, and label the precipitate B.

Exactly neutralise the clear filtrate (from B) with KHO, a precipitate of albumin which is soluble in acids is formed. Filter off precipitate, and label it C.

LACTOSE.

Test the clear filtrate (from C) by Trommer's test (*KHO and* $CuSO_4$) for sugar.

To precipitate A in a test tube add nitric acid, the precipitate is dissolved; boil, and when cold add strong Ammonia, the solution becomes orange-coloured (*xanthoproteic test*).

To a second portion of precipitate A add sodium phosphate, the precipitate will be dissolved; add dilute acetic acid to the solution till a neutral reaction is just obtained, no precipitate occurs; add more acetic acid, a precipitate is thrown down, *casein or alkali albumin is present.*

Test the precipitate on filters B and C by boiling with *Millon's reagent*, a pink coloration will in each case be produced, showing that albumin is present.

PROTEIDS—(*continued*).

CLASS III.—GLOBULINS.

Insoluble in water: *soluble in very dilute acids and alkalies*, soluble in 1°/$_0$ solution of NaCl and other neutral salts.

Differ from native albumins in not being soluble in distilled water, and from derived albumins in being soluble in neutral saline solutions.

Are converted by acids and alkalies into acid—and alkali—albumin respectively.

(1) Globulin or Crystallin.

Obtained from the crystalline lens.

(2) Myosin.

Prepared from dead muscle by removing all fat, tendon, etc., and washing repeatedly in water, until the washing contains no trace of proteids, and then treating with 10°/$_0$ solution of NaCl which will dissolve a large portion into a viscid fluid, which filters with difficulty.

If the viscid filtrate be dropped little by little into a large quantity of distilled water, a white flocculent precipitate of myosin will occur.

(3) Fibrinoplastic Globulin, or Paraglobulin.

Blood serum is diluted with 10 vols. of water, and CO_2 is passed rapidly through, the fine precipitate is collected on a filter, and washed with water containing CO_2.

[Also by saturating serum with NaCl or $MgSO_4$.]

Very soluble in dilute saline solutions, from which it is precipitated by CO_2, and dilute acids $\frac{1}{10}$°/$_0$.

Its solution coagulated at 70°C.

Even dilute acids and alkalies convert it into acid or alkali albumin.

Can be used to form fibrin.

(4) Fibrinogen.

General reactions similar to paraglobulin.

Preparation by similar process from hydrocele fluid.

Its characteristic property is that, when mixed with paraglobulin, it gives fibrin.

(5) Vitellin.

Obtained from yolk of egg.

(6) Globin.

The proteid residue of hæmoglobin.

CLASS IV.—FIBRIN.

A soft white fibrous, and very elastic substance, obtained from blood-clot by washing with large amount of water.

Differs from all other proteids, in having *a filamentous structure*. Examine with microscope.

Has similar chemical properties to coagulated albumin.

Fibrin formed by combining (i) fibrinoplastic globulin and fibrinogen ; (ii) globulin from blood serum with hydrocele fluid.

CLASS V.—PEPTONES.

Are very soluble in water.

Are not precipitated on adding acids or alkalies.

Are not precipitated on boiling.

Insoluble in alcohol and ether, but are only precipitated with difficulty by alcohol.

Are precipitated by mercuric chloride and lead acetate.

With strong solution of NaHO and a trace of copper sulphate they give *a red colour ;* with excess of the salt a violet colour.

Are very diffusible ; experiment with dialysing apparatus.

Digestive Action of Pepsin.

To some fibrin or albumin in a test tube add a trace of pepsin and a little HCl, ·2°/₀, and warm gently ; in a few minutes filter, and test filtrate for peptones.

APPENDIX TO PROTEIDS.

BLOOD.

Alkaline reaction of blood.

Coagulation retarded by freezing and presence of neutral salts.

EXPERIMENT.

Draw a few drops of blood from the finger into a watch-glass previously cooled in a freezing mixture; no coagulation at freezing temperature. A drop of blood added to a little saturated solution of sulphate of soda in a watch-glass; the blood does not coagulate.

Examine the blood with spectroscope. Characteristic spectrum of oxy-hæmoglobin.

Action of *reducing agents on blood, production of spectrum of reduced hæmoglobin.

Action of carbonic oxide (CO) on the blood, alteration of spectrum, no change by action of reducing agents on it.

TESTS FOR BLOOD.

Formation of hæmin crystals, see p. 20.

For guiaicum test see p. 103.

NITROGENOUS BODIES OTHER THAN PROTEIDS.

GELATIN.

Contains more nitrogen and less carbon than albumin.

It is an amorphous, transparent substance.

It is distinguished from the albumins by not being precipitated by ferrocyanide of potassium and by not being coagulated by heat; ordinary gelatin, however, always contains albumin.

It does not, *if pure*, give the proteid reactions. It does not dialyse. It is insoluble in cold water, but swells up to about six times its volume: it dissolves readily on the addition of very dilute acids or alkalies.

Is soluble in hot water, and *forms a jelly on cooling*, even when only 1°/₀ of gelatin is present.

Is precipitated by tannic acid, mercuric chloride, and alcohol.

Not precipitated by dilute mineral acid, nor by acetic acid.

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* *A convenient form of reducing agent is Stokes' Fluid, which is a solution of ferrous sulphate to which NH_4HO has been added, after the previous addition of sufficient tartaric acid to prevent precipitation.*

BONE.

Consists of an organised matrix of connective tissue which contains gelatin and inorganic salts.

Inorganic salts can be removed by digesting bone in hydrochloric acid.

The gelatinous matter left retains the form of bone. By long boiling in water, it is converted into a solution of gelatin.

When bone is heated, the first action is to decompose the organic matter, leaving a deposit of carbon. On further ignition this carbon burns away, and only inorganic salts (principally calcic phosphate) are left.

Solubility of the inorganic salts in hydrochloric acid.

MUCIN.

Is the characteristic component of mucus.

Preparation from ox-gall, by acidulation with acetic acid and subsequent filtration.

Can also be prepared from ox-gall by precipitation with alcohol (afterwards dissolving in water and again precipitating by means of acetic acid).

Can be obtained from mucus by diluting it with water, filtering, treating the insoluble portion with weak caustic alkali and precipitating the mucus with acetic acid.

Mucin has ropy consistency.

It is precipitated by alcohol and mineral acids, but dissolved by excess of the latter.

Is dissolved by alkalies.

Gives the proteid reaction with Millon's reagent and nitric acid, but not with copper sulphate.

Mercuric chloride and tannic acid give no precipitate with it.

It does not dialyse.

NON-NITROGENOUS COMPOUNDS. (CARBOHYDRATES.)

STARCH $(C_6H_{10}O_5)_n$.

Found in almost all plants.

Is a soft white powder, consisting of rounded granules, having an organised structure.

Size of granules varies according to the plant from which they come.

Starch is insoluble in cold water, in alcohol, and in ether.

On boiling with a large amount of water for some time, it becomes soluble and can be filtered.

Soluble and insoluble starch coloured blue by dilute solutions of iodine.

On heating this compound with water, the colour disappears, but returns on cooling.

Starch is obtained from potatoes, by scraping, and washing the scrapings, or from flour, by washing flour tied up in a bag.

GLYCOGEN ($C_6H_{10}O_5$).

White amorphous powder resembling starch. Is soluble in cold water, *turns brown with iodine.*

Ferments or dilute acids convert it into grape sugar.

It occurs in the liver, and after death is found as grape sugar.

Liver should be boiled with a small amount of water, and the solution should be then filtered and tested for grape sugar.

For method of preparing glycogen, see p. 108.

GRAPE SUGAR ($C_6H_{12}O_6$).

Occurs widely diffused in the vegetable kingdom, occurs in diabetic urine, in the blood, etc.

Is obtained by treating honey with cold alcohol, the grape sugar remaining insoluble.

Is easily soluble in water. Is not nearly so sweet as cane sugar.

Not easily charred by H_2SO_4.

Heated with copper sulphate and KHO, cuprous oxide is precipitated. In making the experiment, to the solution of grape sugar add first the caustic potash, then the copper sulphate drop by drop as long as the precipitate formed readily dissolves on shaking the tube, then heat the solution gently.

Quantitative estimation of grape sugar by Fehling's solution, or by saccharometer.

A solution of grape sugar becomes brown on heating with KHO (Moore's test).

It is converted into carbonic acid and alcohol by yeast. $C_6H_{12}O_6 = 2C_2H_5OH + 2CO_2$.

Starch boiled for some time with water and H_2SO_4 is converted into dextrin (British gum) and into grape sugar; this solution to be tested with iodine, and also with potash and copper sulphate.

Starch converted into grape sugar by saliva.

[To collect saliva, fill the mouth with ether vapour. The saliva to be filtered.]

MILK SUGAR (Lactose, $C_{12}H_{24}O_{12}$).

Important constituent of milk. Is much less soluble in water than grape sugar. Only slightly sweet in taste. *Reduces copper salts like grape sugar.*

Is obtained from milk by adding a few drops of H_2SO_4, warming and filtering off the curd.

Also by diffusion.

Test the solution obtained by both methods for milk sugar.

OILS AND FATS.

Neutral substances. Composition, that of a compound ether. (Glycerin $\underset{H_3}{(C_3H_5O_3)}$ being the alcohol.)

They have a lower sp. gr. than water, from 0·91 to 0·94.

They give a greasy stain on paper. Are insoluble in water. *Easily soluble in ether.*

GLYCERIN $\underset{H_3}{(C_3H_5O_3)}$, a viscid liquid, soluble in water and alcohol, insoluble in ether.

Has a characteristic sweet taste.

Acid potassium sulphate heated with glycerin yields acrolein:

$$C_3H_8O_3 - 2H_2O = C_3H_4O.$$

Olive oil, oleate of glycerin $\underset{C_3H_5}{(3(C_{18}H_{33}O_2))}$, or castor oil, heated in a test tube with water, dissolves on addition of NaHO; glycerin is liberated, and sodium oleate is formed. Both are soluble in water.

Olive oil, heated in a basin with water and PbO and well stirred, glycerin is liberated, and oleate of lead (lead plaster) formed.

OLEIN.		SODA.		SODIUM OLEATE.		GLYCERIN.
$\underset{C_3H_5}{3(C_{18}H_{33}O_2)}$	+	$3NaHO$	=	$\underset{Na_3}{3(C_{18}H_{33}O_2)}$	+	$\underset{H_3}{C_3H_5O_3}$

Fat (stearate of glycerin) heated in a test tube with water, melts, and on addition of caustic alkali saponifies, a stearate of sodium and glycerin forming.

Sodium chloride added to this solution, soap (sodium stearate) separates out.

Acid added to another portion, sodium stearate is decomposed. Stearic acid separates out.

To another portion, add a solution of calcium chloride. Calcium stearate is precipitated.

BILE.

An alkaline fluid, sp. gr. 1,018 to 1,020.

ACTION OF BILE ON OILS AND FATS.

Bile shaken up with oil divides it into very small globules (an emulsion formed).

These can, under very slight pressure, pass through animal membranes ; oil alone cannot.

EXPERIMENT.

Take two filters, wet one with water and the other with bile, and pour a small and equal amount of oil on each. Oil passes slowly through the one wetted with bile, not at all through the other.

* Bile acids, Glycocholic, and Taurocholic.

$(C_{26}H_{43}NO_6)$ $(C_{28}H_{45}NSO_7)$

Both give, *with sulphuric acid and sugar, a red colour.*

TESTS FOR BILE.

Pettenkofer's Test for Bile Salts.—Add a few grains of white sugar to solution of bile in test tube, shake well, add strong sulphuric acid, and cool. A reddish purple colour is produced.

Gmelin's Test for Bile Pigments.—Place a drop of bile on a white plate, and add a drop of strong yellow nitric acid to it. A play of colours is produced. Green, blue, red, violet, and yellow, appear in succession.

ACTION OF PANCREATIC JUICE ON FOOD-STUFFS.

1. *It converts Starch into Grape Sugar.*—Add some aqueous extract of pancreas to starch mucilage in test-tube, warm gently, and test solution for grape sugar.

2. *It emulsionizes Fat.*—Shake up some aqueous extract of pancreas with olive-oil in test-tube, an emulsion is formed.

3. *It decomposes Fats, liberating Fatty Acids.*—If the emulsion be allowed to stand for some time, it becomes acid owing to the liberation of the fatty acid.

4. *It converts Proteids into Peptones.*—To boiled fibrin in test-tube, add some glycerin extract of pancreas, diluted with 1°/$_0$ solution of sodium carbonate, and expose to a temperature of 40°C for an hour ; then filter, neutralize filtrate with acetic acid, and test for peptones. *The main difference between the action of pancreatic juice and the action of gastric juice on proteids, is that part of the peptone formed by the former may be broken up into leucin and tyrosin.**

UREA (CON_2H_4). The most characteristic constituent of urine.

Its properties.—Soluble in alcohol and water.

Crystallizes in transparent four-sided prismatic needles, terminated by one or two oblique facets.

Evaporate solution on a glass slide, and examine with microscope.

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* See Appendix.

Precipitation of urea by mercuric nitrate in absence of NaCl, but not in presence of excess of the salt; also by nitric acid.

To strong solution add concentrated HNO_3, which is free from any trace of nitrous acid. Urea nitrate (CON_2H_4 HNO_3) separates out in the form of six-sided tables. Examine with microscope.

Strong solution of oxalic acid added to urea solution, urea oxalate (CON_2H_4 $C_2H_2O_4$) separates out in the form of tabular or prismatic bundles. Examine with microscope.

URIC ACID ($C_5H_4N_4O_3$).

Insoluble in cold water, very slightly soluble in hot.

Dissolves on addition of caustic alkalies, forming urates.

The lithium urates the most soluble.

Murexide test.—Add a drop of strong HNO_3 to uric acid or a urate; evaporate to dryness over water-bath, or at a temperature not exceeding 40°C. Alloxan ($C_4H_2N_2O_4$) is formed. Add a drop of ammonia solution, and the purple colour of murexide ($C_8H_8N_6O_6$) is produced.

HEALTHY URINE is a perfectly transparent, amber-coloured liquid, with a peculiar, but not disagreeable odour, a bitterish taste, and slight acid reaction.

Sp. gr. varies from 1,015 to 1,025.

Urine tested for urea. Evaporate urine to half its bulk, and add strong HNO_3; impure urea nitrate separates out.

Quantitative estimation of urea. Decomposition by sodium hypobromite (NaBrO), in presence of caustic soda, the nitrogen collected and measured.

$$CON_2H_4 + 3NaBrO + 2NaHO = 3NaBr + 3H_2O + Na_2CO_3 + N_2.$$

MORBID URINE.

ALBUMIN PRESENT.

If the urine be neutral or acid, the albumin is precipitated on boiling.

If alkaline, render slightly acid with HNO_3 or acetic acid, and boil.

Coagulated albumin dissolved by caustic alkalies and reprecipitated by HNO_3.

A more delicate test.—Acidify urine with acetic acid. Mucus will be precipitated if present. Filter, and add ferrocyanide of potassium to clear filtrate. A precipitate will be formed in presence of albumin.

BILE PIGMENT, OR ACID PRESENT.

Adopt Gmelin's test (p. 101).

URIC ACID PRESENT.

Employ the Murexide test (p. 102).

URATES PRESENT.

The deposit dissolves on heating; sometimes reappears on cooling.

Urates dissolve in caustic alkalies.

Uric acid separated on adding strong acids.

Murexide test applied to the deposit.

PHOSPHATES PRESENT.

The phosphates may be in solution, or form a deposit.

If in solution the urine is acid. *On boiling urine, phosphates are deposited, the deposited phosphates being soluble in weak acid.*

Phosphates *insoluble in caustic alkalies.*

OXALATES PRESENT.

The deposit is soluble in HCl, but insoluble in acetic acid. Crystalline form.

SUGAR PRESENT (DIABETES).

Sp. gr. generally high, 1,030 to 1,050.

To small amount of urine, add caustic soda, or potash; then a few drops of copper sulphate. Shake up, and heat. The orange-coloured suboxide of copper (Cu_2O) *is precipitated.*

If no grape sugar be present, or too much copper sulphate be added, a black precipitate of cupric oxide (CuO) is produced.

Quantitative estimation of grape sugar by Fehling's solution (Trommer's test).

Diabetic urine boiled with caustic potash or soda becomes brown (Moore's test).

Estimation of sugar by fermentation.—Take sp. gr. of urine before and after fermentation. Each degree of sp. gr. lost by the urine represents one grain of sugar per ounce of urine (Roberts).

Sugar may also be estimated by adding yeast to urine, and collecting the CO_2 evolved. The CO_2 is a measure of the amount of sugar present.

BLOOD PRESENT.

Examine the deposit formed on standing, with the microscope, for blood corpuscles; add to another portion a drop of tincture of guiaicum, and about a drachm of ozonic ether, a blue colour will appear at the junction of the fluids.

Pus Present.

Examine deposit with the microscope.

Add caustic potash, the urine becomes stringy.

ANALYSIS OF URINARY CALCULI AND DEPOSITS.

If a calculus, ignite a small portion on platinum foil. If it burns away completely, it is probably uric acid. Apply murexide test.

Boil the powdered calculus with distilled water, or, if a urinary deposit, with the supernatant urine, and filter.

INSOLUBLE PORTION.

Phosphates, calcium oxalate, uric acid.

Boil with a few drops of hydrochloric acid, and filter.

Insoluble.	*Soluble.*
Uric acid. Apply murexide test.	Phosphates or calcium oxalate. Add excess of ammonia, and then acetic acid in excess: a precipitate remaining undissolved is calcium oxalate. To the clear liquid, whether filtered or not, add ammonia: a precipitate indicates the presence of phosphates.

SOLUBLE PORTION.

Urates.

Mostly deposit on cooling.

Test for urate of ammonia by boiling with potash to demonstrate the presence of ammonia, and by the murexide test for the uric acid.

APPENDIX.

The injection of blood-vessels and lymphatics can be effected either by means of a simple syringe or by the pressure of a column of air, water, or mercury. These methods are described at length in the "Handbook for the Physiological Laboratory," pp. 97—103 (by Dr. Klein), and elsewhere.

The fluids employed are :—

(*a*) *Berlin blue*, either dissolved or suspended in water, or in solution in gelatin. A 2% solution of the material is chiefly employed.

(*b*) *Carmine*, either dissolved or suspended in water, or in solution in gelatin.

(*c*) *Nitrate of silver* used in a 0·25 or 0·5% solution.

Warm stages of various kinds are in use. The simplest is a glass slide to which a perforated circular plate of copper is attached with cement: this is joined to a projecting rod of the same metal. The rod communicates heat from a spirit lamp to the plate, upon which is placed the specimen of blood to be examined. It is best to use two large cover glasses, arranged as a "moist chamber"; one has upon it the blood to be examined, the other is edged round with oil; when applied to each other the oil prevents the fluid of the blood from evaporating, and under these circumstances the movement of the colourless corpuscles can be watched for some time. The temperature is regulated by placing a small piece of cacao-butter on a flattened portion of the rod near the copper disc. When the butter melts, it is a warning to remove the spirit-lamp.

Stricker's warm stage is more elaborate; it is provided with a thermometer which accurately regulates the temperature. Of this instrument there are several varieties (see "Hdbk. Phys. Laboratory," p. 7, and plates). It is usually arranged so that gases can be introduced to act upon the blood under examination.

Action of Gases on the Blood.—The warm stage is to be used, a ring of putty being placed round the central chamber. A drop of blood diluted with 0·75% saline solution is placed on a cover glass, which is then inverted upon the putty. The chamber thus becomes air-tight, and should be kept moist by placing a drop of water in it. The stage is then put into communication with the apparatus used for generating the required gas by means of indiarubber tubing.

Action of Carbonic Acid Gas.—The preparation is brought into focus, and the gas is allowed to pass through the chamber. *Human.*—The red blood-corpuscles, which had become crenate from the action of the salt solution, again acquire a smooth outline, owing to the swelling up of the parts between the projections. On admitting air to the chamber the corpuscles again become crenate. The nuclei in the white corpuscles become more distinct. *Amphibia.*—The nuclei in the red corpuscles appear more distinct, owing to the coagulation of the substance surrounding the nucleus.

Action of Chloroform on the Blood.—Preparation as for gases. The red corpuscles become globular, the hæmoglobin being finally dissolved and discharged into the plasma; the blood, when seen with the naked eye, being transparent (lake) and no longer opaque.

Action of Electricity on the Blood.—The blood is placed on a slide in such a position that when it is covered it spreads between two poles of tinfoil situated six millimetres apart, which are connected with the secondary coil of an induction apparatus. After a succession of *induction* shocks the *red corpuscles* lose their smooth outline, become crenate, then like mulberries, and finally horsechestnut-shaped. They swell up, and ultimately become decolorised. The *white corpuscles* swell up and disintegrate, their granules exhibiting molecular movements. With a *constant current* from a single Bunsen's cell the *red* corpuscles at the positive pole undergo changes which correspond to those exhibited under the action of an alkali, and at the negative pole of an acid. The *colourless* corpuscles assume a spheroidal form, the amœboid movements being resumed as soon as the stimulus has passed.

The Action of Alkalies on Blood.—A mixture of 2cc. of caustic potash in 1000cc. of saline solution causes both red and white blood-corpuscles to swell up, burst, and disappear. The red corpuscles appear to be more rapidly affected than the white.

The Action of Alcohol.—A mixture of one-third spirit and two-thirds water, acting upon amphibian blood, causes the nucleus to swell, and brings into view the nucleolus in the red corpuscles. It also renders the nuclei of the colourless corpuscles more evident, whilst one or more delicate and clear "blebs" grow from the periphery of the white corpuscle. These "blebs" appear to consist of some colloid substance, into which endosmosis rapidly takes place. (*Rutherford.*)

The Method of Feeding the Colourless Corpuscles.—The preparation of blood on the warm stage is irrigated with carmine, vermilion, or anilin blue, in a finely divided state (for method of preparation see "Handbook for the Physiological Laboratory," p. 10), or with milk. The white corpuscles will be found after a short time to have ingested some of the finer particles.

The particles are taken into the substance of the corpuscle by the union around it of two of the protoplasmic processes, and they thus lie at first close to the periphery of the cell, being carried at a later period nearer to its centre.

Ciliary Motion.—The epithelium scraped from the roof of a frog's mouth, or epithelium obtained from the mucus of the nose, may be employed. The gills of the ordinary mussel or oyster, and the epithelium lining the alimentary canal of the earthworm, are also well adapted for the demonstration of ciliary motion. The examination is to be made in normal saline solution, the preparation being slightly teased previous to covering. The highest available power should be used.

Effects of Reagents.—*Dilute alkalies* slow, and then stop the movements. If the cilia are working slowly, or have stopped in a preparation which has just been put up, the careful addition of a very dilute solution of caustic potash or dilute acetic acid, or the passage over it of carbonic acid, or an electric shock, will generally renew or accelerate the movements for a short time,—the ultimate effect, however, being to destroy the cilia. *Carbonic acid* first accelerates, then slows, and finally stops the ciliary action, the movements recommencing if air is allowed to take the place of the carbonic acid.

Chloroform retards and finally stops ciliary action; the movements recommencing on the admission of air, if the vapour has not been applied for too long a period.

Warmth accelerates the action of cilia which were previously moving slowly, the movements ceasing at a temperature which is sufficient to destroy the vitality of the cells.

Preparation of Glycogen.—Apparatus necessary. A solution of potassic-mercuric iodide made by precipitating a solution of mercuric chloride with potassium iodide, washing the precipitate and adding it to a boiling solution of potassium iodide till the latter is saturated. Any precipitate which occurs on cooling is to be filtered off. Dilute hydrochloric acid. Methylated spirit, a large bottle; ether; absolute alcohol. Large filter and Swedish filter papers. Large knife; capsule; several beakers; distilled water; ice. Mortar and pestle; large Bunsen's burner.

Glycogen, usually obtained from the liver of animals, is also present to a considerable extent in the muscles of very young animals. To prepare glycogen, it is best to use the liver of a rabbit. The animal should be large, and it must have been well fed on a diet of grain and sugar for some days, preferably weeks, previously. The rabbit should have a full meal of grain and sugar about two hours before it is killed, in order that it may be in full digestion. Before destroying the animal the capsule is to be filled with water, which is kept briskly boiling by means of the large Bunsen's burner. The rabbit is killed either by decapitation or by a blow on the head, and the abdomen is then rapidly opened, and the liver is torn out, is chopped up as quickly as possible with the knife, and is thrown into the boiling water. It is important that this operation should be performed within half a minute of the death of the animal, and that the water should not be allowed to fall below the boiling-point. The liver is to remain in the capsule for five minutes; it is then poured into a mortar, the liquid being returned to the capsule. The liver is then reduced to a pulp, and is again boiled in the capsule for ten minutes. The liquid is filtered, and the filtrate is rapidly cooled by placing the vessel in iced water. The albuminous substances in the cold filtrate are precipitated by adding potassio-mercuric iodide and dilute hydrogen chloride alternately as long as any precipitate is produced. (The albumin may also be destroyed by boiling in a strong solution of sodium sulphate.) The mixture is then stirred, is allowed to stand for five minutes, and is filtered. Alcohol is added to this second filtrate until glycogen is precipitated (*i.e.*, until about 60% of absolute alcohol has been added. The precipitate is then filtered off, and is washed with weak spirit, strong spirit, absolute alcohol (two or three times), and finally with ether. It is then dried on a glass plate at a moderate heat, and if pure should remain as a white amorphous powder. If the water has not been completely removed, the glycogen will form a gummy mass ; in this case it must be again treated with absolute alcohol.

Preparation of Leucin and Tyrosin.—These bodies may be obtained by digesting fibrin for ten to twelve hours with pancreatic juice. The albumin is precipitated by slightly acidulating, boiling and filtering the solution. The filtrate is then evaporated to a small bulk, and heated with strong alcohol to precipitate the peptones. On again filtering, an extract is obtained from which leucin and tyrosin crystallise. The two bodies can be separated from each other by the addition of boiling alcohol, in which leucin is soluble, and from which it can be recrystallised. (*Burdon Sanderson.*)

Preparation of Bilin.—Mix bile, which has been evaporated to one-fourth its bulk, with animal charcoal, evaporate to perfect dryness on a water-bath, and extract it, whilst still warm, with absolute alcohol. The alcoholic filtrate should be colourless; if this is not the case, more charcoal must be added. The alcohol is distilled off, and the dry residue is treated with absolute alcohol. The alcohol is then filtered off, and to the filtrate anhydrous ether is added as long as a precipitate is thrown down. The solution and precipitate are to be set aside in a closely-stoppered bottle for some days, when crystals of bilin will be produced. If the reagents were not perfectly anhydrous, a gelatinous mass will be formed, but no crystals. Bilin consists of glycocholic and taurocholic acids, which may be separated by dissolving the bilin in water, and adding first solution of neutral lead acetate and then a little basic lead acetate. This combines with the glycocholic acid, and forms an insoluble lead glycocholate. Filter, and add to the filtrate lead acetate and ammonia, and a precipitate of lead taurocholate will be formed, which may be filtered off. In either case the lead may be got rid of by suspending or dissolving in hot alcohol, adding hydrogen sulphide, and filtering.

Preparation of Cholesterin.—Pulverised gall stones are extracted with boiling alcohol, and the extract is filtered whilst boiling. Crystals of cholesterin separate out from the filtrate when cool. These may be purified by boiling with an alcoholic solution of caustic potash, washing with cold alcohol, and then with water; dissolving in a mixture of alcohol and ether, and allowing it to evaporate.

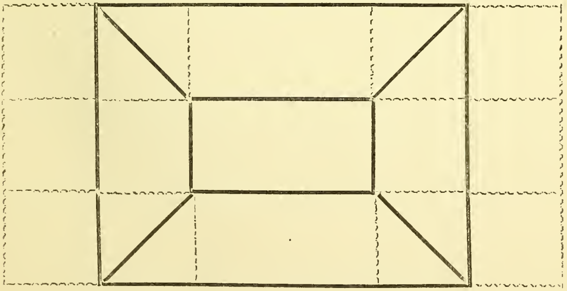
DEVELOPMENT OF TEETH.

Preparation.—The fœtus of a new-born rat is decapitated, and its head is placed in a large excess of $\frac{1}{6}\%$ chromic acid for a week; it is then transferred to spirit. The lower jaw may then be removed, and embedded in the ordinary way; the sections should be stained in hæmatoxylin and in carmine.

(1) The first rudiment of a tooth appears as a solid prolongation of the stratified epithelium, which grows downwards from the surface into the mucous membrane. This process of epithelium is the *primary enamel organ.* (2) The enamel organ becomes invaginated at its deep end by a mass of tissue derived from the mucous membrane, called the embryonal tooth papilla. The primary enamel organ is thus converted into the *enamel cap* covering the tooth papilla. (3) *The papilla* is vascular and is composed of a network of nucleated cells; it forms the pulp, and by means of its odontoblasts forms the dentine. (4) *The Odontoblasts* appear on the papilla as a peripheral stratum of large cells arranged vertically. (5) *The dentine* is formed by the elongation and subsequent calcification of the distal extremities of the odontoblasts, whilst (6) *the dentinal fibres* are derived from processes of cells wedged in between the odontoblasts. (7) *The tooth sac*, or the mucous membrane which immediately surrounds the enamel cap and tooth papilla, gradually grows over the former, and separates it from its connection with the surface epithelium. (8) The *enamel cap* consists externally of (*a*) columnar cells, more internally of (*b*) polyhedral cells, followed by (*c*) flattened epithelial cells in the centre, and again of (*d*) polyhedral, with (*e*) columnar cells most internally—*i.e.*, nearest to the tooth papilla. (9) The enamel cap is limited both externally and internally by a *membrana propria.* (10) The enamel cap becomes divided into an inner and outer membrane by the transformation of the middle layer (8*c*) into a transparent tissue.

The inner membrane is composed of columnar cells, the enamel cells, in contact with the dentine; each is a long hexagonal prism, and is nucleated at its lower part. Outside the layers of enamel cells are one or more rows of small polyhedral cells, forming the stratum intermedium. *The outer membrane* is composed of several layers of epithelial cells. (11) *The enamel* is formed by the enamel cells of the inner membrane elongating at their distal extremities; the elongated portion is transformed rapidly into enamel. (12) The cells of the stratum intermedium are used for the regeneration of the enamel. (13) The cells of the outer epithelium produce the *enamel cuticle.* (14) *The cement* is formed from the tissue of the tooth sac in exactly the same way as sub-periosteal bone is developed. (15) During the stage of the primary enamel organ (i) a lateral process grows out from the epithelial cells, which represents the rudiment of the enamel organ of the permanent tooth (*sac of reserve*). (16) *The Permanent* teeth are developed on exactly the same plan as the deciduous set. (Klein.)

Paper "boat" for Embedding in Wax may be made by taking a piece of stout paper, six inches long and three broad, and doubling it into three folds lengthwise, and then doubling down flaps of two inches from each end. The paper is then opened out, and the lateral folds form the sides of the boat. To arrange the ends, each flap is marked off into two equal parts, one inch of which is for the end, and the other, after the corners are arranged, forms an external flap to keep the folding necessary for this in place.



Classification of Staining Fluids.—Mr. J. W. Groves, in an excellent paper read before the Quekett Microscopical Club, gives the following classification of staining fluids :—

- *A.* General stains.
  - Carmine with excess of ammonia.
  - Eosin (Dreschfeld).
  - Molybdate of ammonia. (This requires the action of light.)
- *B.* Selective stains.
  - Simple. Not requiring the action of light.
    - Carmine (Beale).
    - Borax carmine (Golding Bird).
    - Logwood (Golding Bird).
    - Logwood acid solution (Schäfer).
    - Indigo carmine (Tiersch).
    - Anilin blue (Heidenhain).
    - Picric acid.
  - Simple. Requiring the action of light.
    - Gold chloride.
    - Silver nitrate.
    - Osmic acid.
    - Chloride of palladium.
  - Double.
    - Molybdate of ammonia and carmine.
    - Picrocarmin (Schäfer).
    - Chloride of palladium and carmine.
    - Carmine and indigo carmine.
    - Logwood and anilin blue.
    - Gold chloride and logwood.
    - Silver nitrate and logwood.
    - Silver nitrate and gold chloride.
- *C.* Which will stain in the mass and harden at the same time.
  - Osmic acid.
  - Picric acid.
  - Gold chloride.
  - Alcohol + borax carmine.
  - Alcohol + Golding Bird's logwood.
  - Alcohol + eosin.

Magnifying Powers of Microscopes.—Messrs. Parkes furnish the following table of the magnifying power of some of their microscopes, which may be taken as a type of the ordinary instruments :—

		Approximate magnifying power with Eyepieces :			
		A	B	C	
Focal length.		Diameters.	Diameters.	Diameters.	Angular aperture.
1 inch	Draw-tube closed	65	95	145	18°
	Ditto if drawn out, add for each inch	13	19	29	
$\frac{1}{3}$ inch	Draw-tube closed	100	147	225	30°
	Ditto if drawn out, add for each inch	20	30	45	
$\frac{1}{2}$ inch	Draw-tube closed	125	183	280	35°
	Ditto if drawn out add for each inch	25	37	56	
$\frac{1}{4}$ inch	Draw-tube closed	260	380	580	70°
	Ditto if drawn out, add for each inch	52	76	116	
$\frac{1}{6}$ inch	Draw-tube closed	333	486	743	95°
	Ditto if drawn out, add for each inch	66	97	148	

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